

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
6 July 2000 (06.07.2000)

PCT

(10) International Publication Number  
**WO 00/39304 A2**

- (51) International Patent Classification<sup>7</sup>: C12N 15/49, A61K 48/00
- (21) International Application Number: PCT/US99/31273
- (22) International Filing Date:  
30 December 1999 (30.12.1999)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/114,495 31 December 1998 (31.12.1998) US  
60/152,195 1 September 1999 (01.09.1999) US
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- (81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— Without international search report and to be republished upon receipt of that report.
- (48) Date of publication of this corrected version:  
7 December 2000
- (15) Information about Correction:  
see PCT Gazette No. 49/2000 of 7 December 2000, Section II

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

A2  
WO 00/39304

(54) Title: POLYNUCLEOTIDES ENCODING ANTIGENIC HIV TYPE C POLYPEPTIDES, POLYPEPTIDES AND USES THEREOF

(57) Abstract: The present invention relates to polynucleotides encoding immunogenic HIV type C Gag- and/or Env-containing polypeptides. Uses of the polynucleotides in applications including DNA immunization, generation of packaging cell lines, and production of Gag- and/or Env-containing proteins are also described.

**POLYNUCLEOTIDES ENCODING ANTIGENIC HIV TYPE C POLYPEPTIDES,  
POLYPEPTIDES AND USES THEREOF**

**TECHNICAL FIELD**

5 Polynucleotides encoding antigenic Type C HIV Gag-containing polypeptides and/or Env-containing polypeptides are described, as are uses of these polynucleotides and polypeptide products in immunogenic compositions.

**BACKGROUND OF THE INVENTION**

10 Acquired immune deficiency syndrome (AIDS) is recognized as one of the greatest health threats facing modern medicine. There is, as yet, no cure for this disease.

In 1983-1984, three groups independently identified the suspected etiological agent of AIDS. See, e.g., Barre-Sinoussi et al. (1983) Science 220:868-871; Montagnier et 15 al., in Human T-Cell Leukemia Viruses (Gallo, Essex & Gross, eds., 1984); Vilmer et al. (1984) The Lancet 1:753; Popovic et al. (1984) Science 224:497-500; Levy et al. (1984) Science 225:840-842. These isolates were variously called lymphadenopathy-associated virus (LAV), human T-cell lymphotropic virus type III (HTLV-III), or AIDS-associated retrovirus (ARV). All of these isolates are strains of 20 the same virus, and were later collectively named Human Immunodeficiency Virus (HIV). With the isolation of a related AIDS-causing virus, the strains originally called HIV are now termed HIV-1 and the related virus is called HIV-2 See, e.g., Guyader et al. (1987) Nature 326:662-669; Brun-Vezinet et al. (1986) Science 233:343-346; Clavel et al. (1986) Nature 324:691-695.

25 A great deal of information has been gathered about the HIV virus, however, to date an effective vaccine has not been identified. Several targets for vaccine development have been examined including the *env* and *Gag* gene products encoded by HIV. *Gag* gene products include, but are not limited to, *Gag*-polymerase and *Gag*-

protease. Env gene products include, but are not limited to, monomeric gp120 polypeptides, oligomeric gp140 polypeptides and gp160 polypeptides.

Haas, et al., (*Current Biology* 6(3):315-324, 1996) suggested that selective codon usage by HIV-1 appeared to account for a substantial fraction of the 5 inefficiency of viral protein synthesis. Andre, et al., (*J. Virol.* 72(2):1497-1503, 1998) described an increased immune response elicited by DNA vaccination employing a synthetic gp120 sequence with optimized codon usage. Schneider, et al., (*J Virol.* 71(7):4892-4903, 1997) discuss inactivation of inhibitory (or instability) elements (INS) located within the coding sequences of the Gag and Gag-protease coding 10 sequences.

The *Gag* proteins of HIV-1 are necessary for the assembly of virus-like particles. HIV-1 *Gag* proteins are involved in many stages of the life cycle of the virus including, assembly, virion maturation after particle release, and early post-entry steps in virus replication. The roles of HIV-1 *Gag* proteins are numerous and 15 complex (Freed, E.O., *Virology* 251:1-15, 1998).

Wolf, et al., (PCT International Application, WO 96/30523, published 3 October 1996; European Patent Application, Publication No. 0 449 116 A1, published 2 October 1991) have described the use of altered pr55 *Gag* of HIV-1 to act as a non-infectious retroviral-like particulate carrier, in particular, for the presentation of 20 immunologically important epitopes. Wang, et al., (*Virology* 200:524-534, 1994) describe a system to study assembly of HTV Gag- $\beta$ -galactosidase fusion proteins into virions. They describe the construction of sequences encoding HIV Gag- $\beta$ -galactosidase fusion proteins, the expression of such sequences in the presence of HIV Gag proteins, and assembly of these proteins into virus particles.

25 Shiver, et al., (PCT International Application, WO 98/34640, published 13 August 1998) described altering HIV-1 (CAM1) *Gag* coding sequences to produce synthetic DNA molecules encoding HIV *Gag* and modifications of HIV *Gag*. The codons of the synthetic molecules were codons preferred by a projected host cell.

Recently, use of HIV Env polypeptides in immunogenic compositions has 30 been described. (see, U.S. Patent No. 5,846,546 to Hurwitz et al., issued December 8, 1998, describing immunogenic compositions comprising a mixture of at least four different recombinant virus that each express a different HIV env variant; and U.S.

Patent No. 5,840,313 to Vahlne et al., issued November 24, 1998, describing peptides which correspond to epitopes of the HIV-1 gp120 protein). In addition, U.S. Patent No. 5,876,731 to Sia et al, issued March 2, 1999 describes candidate vaccines against HIV comprising an amino acid sequence of a T-cell epitope of Gag linked directly to 5 an amino acid sequence of a B-cell epitope of the V3 loop protein of an HIV-1 isolate containing the sequence GPGR. There remains a need for antigenic HIV polypeptides, particularly Type C isolates.

#### SUMMARY OF THE INVENTION

10 The present invention relates to improved expression of HIV Type C *Gag*-containing polypeptides and production of virus-like particles, as well as, *Env*-containing polypeptides.

One aspect of the present invention relates to expression cassettes and polynucleotides contained therein. In one embodiment, an expression cassette 15 comprises a polynucleotide sequence encoding a polypeptide including an HIV *Gag*-containing polypeptide, wherein the polynucleotide sequence encoding the *Gag* polypeptide comprises a sequence having at least about 85%, preferably about 90%, more preferably about 95%, and most preferably about 98% sequence identity to the sequences taught in the present specification. The polynucleotide sequences encoding 20 *Gag*-containing polypeptides include, but are not limited to, the following polynucleotides: nucleotides 844-903 of Figure 1 (a *Gag* major homology region) (SEQ ID NO:1); nucleotides 841-900 of Figure 2 (a *Gag* major homology region) (SEQ ID NO:2); the sequence presented as Figure 1 (SEQ ID NO:3); and the sequence presented as Figure 2 (SEQ ID NO:4). The polynucleotides encoding the 25 *Gag*-containing polypeptides of the present invention may also include sequences encoding additional polypeptides. Such additional polynucleotides encoding polypeptides may include, for example, coding sequences for other HIV proteins, such as, polynucleotide sequences encoding an HIV *protease* polypeptide, and polynucleotide sequences encoding an HIV *polymerase* polypeptide. In one 30 embodiment, the sequence encoding the HIV *polymerase* polypeptide can be modified by deletions of coding regions corresponding to reverse transcriptase and integrase. Such deletions in the polymerase polypeptide can also be made such that the

polynucleotide sequence preserves T-helper cell and CTL epitopes. Other antigens of interest may be inserted into the polymerase as well.

- In another embodiment, an expression cassette comprises a polynucleotide sequence encoding a polypeptide including an HIV *Env*-containing polypeptide,
- 5 wherein the polynucleotide sequence encoding the *Gag* polypeptide comprises a sequence having at least about 85%, preferably about 90%, more preferably about 95%, and most preferably about 98% sequence identity to the sequences taught in the present specification. The polynucleotide sequences encoding *Env*-containing polypeptides include, but are not limited to, the following polynucleotides:
- 10 nucleotides 1213-1353 of Figure 3 (SEQ ID NO:5) (an Env common region); nucleotides 82-1512 of Figure 3 (SEQ ID NO:6) (a gp120 polypeptide); nucleotides 82-2025 of Figure 3 (SEQ ID NO:7) (a gp140 polypeptide); nucleotides 82-2547 of Figure 3 (SEQ ID NO:8) (a gp160 polypeptide); nucleotides 1-2547 of Figure 3 (SEQ ID NO:9) (a gp160 polypeptide with signal sequence); nucleotides 1513-2547 of Figure 3 (SEQ ID NO:10) (a gp41 polypeptide); nucleotides 1210-1353 of Figure 4 (SEQ ID NO:11) (an Env common region); nucleotides 73-1509 of Figure 4 (SEQ ID NO:12) (a gp120 polypeptide); nucleotides 73-2022 of Figure 4 (SEQ ID NO:13) (a gp140 polypeptide); nucleotides 73-2565 of Figure 4 (SEQ ID NO:14) (a gp160 polypeptide); nucleotides 1-2565 of Figure 4 (SEQ ID NO:15) (a gp160 polypeptide with signal sequence); and nucleotides 1510-2565 of Figure 4 (SEQ ID NO:16) (a gp41 polypeptide).
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- 20
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The present invention further includes recombinant expression systems for use in selected host cells, wherein the recombinant expression systems employ the polynucleotides and expression cassettes of the present invention. In such systems, the polynucleotide sequences are operably linked to control elements compatible with expression in the selected host cell. Numerous expression control elements are known to those in the art, including, but not limited to, the following: transcription promoters, transcription enhancer elements, transcription termination signals, polyadenylation sequences, sequences for optimization of initiation of translation, and translation termination sequences. Exemplary transcription promoters include, but are not limited to those derived from CMV, CMV+intron A, SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

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In another aspect the invention includes cells comprising the expression cassettes of the present invention where the polynucleotide sequence (e.g., encoding an Env- and/or Gag-containing polypeptide) is operably linked to control elements compatible with expression in the selected cell. In one embodiment such cells are

5 mammalian cells. Exemplary mammalian cells include, but are not limited to, BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells. Other cells, cell types, tissue types, etc., that may be useful in the practice of the present invention include, but are not limited to, those obtained from the following: insects (e.g., *Trichoplusia ni* (Tn5) and Sf9), bacteria, yeast, plants, antigen presenting cells (e.g., macrophage,

10 monocytes, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), primary cells, immortalized cells, tumor-derived cells.

In a further aspect, the present invention includes compositions for generating an immunological response, where the composition typically comprises at least one of the expression cassettes of the present invention and may, for example, contain

15 combinations of expression cassettes (such as one or more expression cassettes carrying a Gag-polypeptide-encoding polynucleotide and one or more expression cassettes carrying an Env-polypeptide-encoding polynucleotide). Such compositions may further contain an adjuvant or adjuvants. The compositions may also contain one or more Gag-containing polypeptides and/or one or more Env-containing

20 polypeptides. The Gag-containing polypeptides and/or Env-containing polypeptides may correspond to the polypeptides encoded by the expression cassette(s) in the composition, or, the Gag-containing polypeptides and/or Env-containing polypeptides may be different from those encoded by the expression cassettes. An example of the polynucleotide in the expression cassette encoding the same polypeptide as is being

25 provided in the composition is as follows: the polynucleotide in the expression cassette encodes the Gag-polypeptide of Figure 1 (SEQ ID NO:3), and the polypeptide is the polypeptide encoded by the sequence shown in Figure 1 (SEQ ID NO:17). An example of the polynucleotide in the expression cassette encoding a different polypeptide as is being provided in the composition is as follows: an

30 expression cassette having a polynucleotide encoding a Gag-polymerase polypeptide, and the polypeptide provided in the composition may be a Gag and/or Gag-protease polypeptide. In compositions containing both expression cassettes (or

polynucleotides of the present invention) and polypeptides, the Env and Gag expression cassettes of the present invention can be mixed and/or matched with Env-containing and Gag-containing polypeptides described herein.

In another aspect the present invention includes methods of immunization of a subject. In the method any of the above described compositions are into the subject under conditions that are compatible with expression of the expression cassette in the subject. In one embodiment, the expression cassettes (or polynucleotides of the present invention) can be introduced using a gene delivery vector. The gene delivery vector can, for example, be a non-viral vector or a viral vector. Exemplary viral vectors include, but are not limited to Sindbis-virus derived vectors, retroviral vectors, and lentiviral vectors. Compositions useful for generating an immunological response can also be delivered using a particulate carrier. Further, such compositions can be coated on, for example, gold or tungsten particles and the coated particles delivered to the subject using, for example, a gene gun. The compositions can also be formulated as liposomes. In one embodiment of this method, the subject is a mammal and can, for example, be a human.

In a further aspect, the invention includes methods of generating an immune response in a subject, wherein the expression cassettes or polynucleotides of the present invention are expressed in a suitable cell to provide for the expression of the Env- and/or Gag-containing polypeptides encoded by the polynucleotides of the present invention. The polypeptide(s) are then isolated (e.g., substantially purified) and administered to the subject in an amount sufficient to elicit an immune response.

The invention further includes methods of generating an immune response in a subject, where cells of a subject are transfected with any of the above-described expression cassettes or polynucleotides of the present invention, under conditions that permit the expression of a selected polynucleotide and production of a polypeptide of interest (e.g., encoded by any expression cassette of the present invention). By this method an immunological response to the polypeptide is elicited in the subject. Transfection of the cells may be performed *ex vivo* and the transfected cells are reintroduced into the subject. Alternately, or in addition, the cells may be transfected *in vivo* in the subject. The immune response may be humoral and/or cell-mediated (cellular). In a further embodiment, this method may also include administration of

an Env- and/or Gag-containing polypeptide before, concurrently with, and/or after introduction of the expression cassette into the subject.

Further embodiments of the present invention include purified polynucleotides. Exemplary polynucleotide sequences encoding *Gag*-containing polypeptides include, but are not limited to, the following polynucleotides: nucleotides 844-903 of Figure 1 (SEQ ID NO:1) (a *Gag* major homology region); nucleotides 841-900 of Figure 2 (SEQ ID NO:2) (a *Gag* major homology region); the sequence presented as Figure 1 (SEQ ID NO:3); and the sequence presented as Figure 2 (SEQ ID NO:4). Exemplary polynucleotide sequences encoding *Env*-containing polypeptides include, but are not limited to, the following polynucleotides: nucleotides 1213-1353 of Figure 3 (SEQ ID NO:5) (an *Env* common region); nucleotides 82-1512 of Figure 3 (SEQ ID NO:6) (a gp120 polypeptide); nucleotides 82-2025 of Figure 3 (SEQ ID NO:7) (a gp140 polypeptide); nucleotides 82-2547 of Figure 3 (SEQ ID NO:8) (a gp160 polypeptide); nucleotides 1-2547 of Figure 3 (SEQ ID NO:9) (a gp160 polypeptide with signal sequence); nucleotides 1513-2547 of Figure 3 (SEQ ID NO:10) (a gp41 polypeptide); nucleotides 1210-1353 of Figure 4 (SEQ ID NO:11) (an *Env* common region); nucleotides 73-1509 of Figure 4 (SEQ ID NO:12) (a gp120 polypeptide); nucleotides 73-2022 of Figure 4 (SEQ ID NO:13) (a gp140 polypeptide); nucleotides 73-2565 of Figure 4 (SEQ ID NO:14) (a gp160 polypeptide); nucleotides 1-2565 of Figure 4 (SEQ ID NO:15) (a gp160 polypeptide with signal sequence); and nucleotides 1510-2565 of Figure 4 (SEQ ID NO:16) (a gp41 polypeptide). The polynucleotide sequence encoding the *Gag*-containing and *Env*-containing polypeptides of the present invention typically have at least about 85%, preferably about 90%, more preferably about 95%, and most preferably about 98% sequence identity to the sequences taught herein.

The polynucleotides of the present invention can be produced by recombinant techniques, synthetic techniques, or combinations thereof.

These and other embodiments of the present invention will readily occur to those of ordinary skill in the art in view of the disclosure herein.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 (SEQ ID NO:3) shows the nucleotide sequence of a polynucleotide encoding a synthetic Gag polypeptide. The nucleotide sequence shown was obtained by modifying type C strain AF110965 and include further modifications of INS.

5 Figure 2 (SEQ ID NO: 4) shows the nucleotide sequence of a polynucleotide encoding a synthetic Gag polypeptide. The nucleotide sequence shown was obtained by modifying type C strain AF110967 and include further modifications of INS.

10 Figure 3 (SEQ ID NO:9) shows the nucleotide sequence of a polynucleotide encoding a synthetic Env polypeptide. The nucleotide sequence depicts gp160 (including a signal peptide) and was obtained by modifying type C strain AF110968. The arrows indicate the positions of various regions of the polynucleotide, including the sequence encoding a signal peptide (nucleotides 1-81) (SEQ ID NO:18), a gp120 polypeptide (nucleotides 82-1512) (SEQ ID NO:6), a gp41 polypeptide (nucleotides 1513-2547) (SEQ ID NO:10), a gp140 polypeptide (nucleotides 82-2025) (SEQ ID  
15 NO:7) and a gp160 polypeptide (nucleotides 82-2547) (SEQ ID NO:8). The codons encoding the signal peptide are modified (as described herein) from the native HIV-1 signal sequence.

20 Figure 4 (SEQ ID NO:15) shows the nucleotide sequence of a polynucleotide encoding a synthetic Env polypeptide. The nucleotide sequence depicts gp160 (including a signal peptide) and was obtained by modifying type C strain AF110975. The arrows indicate the positions of various regions of the polynucleotide, including the sequence encoding a signal peptide (nucleotides 1-72) (SEQ ID NO:19), a gp120 polypeptide (nucleotides 73-1509) (SEQ ID NO:12), a gp41 polypeptide (nucleotides 1510-2565) (SEQ ID NO:16), a gp140 polypeptide (nucleotides 73-2022) (SEQ ID  
25 NO:13), and a gp160 polypeptide (nucleotides 73-2565) (SEQ ID NO:14). The codons encoding the signal peptide are modified (as described herein) from the native HIV-1 signal sequence.

30 Figure 5 shows the location of some remaining INS in synthetic Gag sequences derived from AF110965. The changes made to these sequences are boxed in the Figures. The top line depicts a codon optimized sequence of Gag polypeptides from the indicated strains (SEQ ID NO:20). The nucleotide(s) appearing below the

line in the boxed region(s) depicts changes made to remove further INS and correspond to the sequence depicted in Figure 1 (SEQ ID NO:3).

- Figure 6 shows the location of some remaining INS in synthetic Gag sequences derived from AF110968. The changes made to these sequences are boxed
- 5      in the Figures. The top line depicts a codon optimized sequence of Gag polypeptides from the indicated strains (SEQ ID NO:21). The nucleotide(s) appearing below the line in the boxed region(s) depicts changes made to remove further INS and correspond to the sequence depicted in Figure 2 (SEQ ID NO:4).

## 10      DETAILED DESCRIPTION OF THE INVENTION

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, molecular biology, immunology and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); *Methods In Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); and *Handbook of Experimental Immunology*, Vols. I-IV (D.M. Weir and C.C. Blackwell, eds., 1986, Blackwell Scientific Publications); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Short Protocols in Molecular Biology*, 4th ed. (Ausubel et al. eds., 1999, John Wiley & Sons); *Molecular Biology Techniques: An Intensive Laboratory Course*, (Ream et al., eds., 1998, Academic Press); *PCR (Introduction to Biotechniques Series)*, 2nd ed. (Newton & Graham eds., 1997, Springer Verlag).

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural references unless the content clearly dictates otherwise.

25      Thus, for example, reference to "an antigen" includes a mixture of two or more such agents.

### 1. DEFINITIONS

In describing the present invention, the following terms will be employed, and

30      are intended to be defined as indicated below.

"Synthetic" sequences, as used herein, refers to Gag-encoding polynucleotides whose expression has been optimized as described herein, for example, by codon substitution and inactivation of inhibitory sequences. "Wild-type" or "native" sequences, as used herein, refers to polypeptide encoding sequences that are essentially as they are found in nature, e.g., Gag and/or Env encoding sequences as found in Type C isolates, e.g., AF110965, AF110967, AF110968 or AF110975.

As used herein, the term "virus-like particle" or "VLP" refers to a nonreplicating, viral shell, derived from any of several viruses discussed further below. VLPs are generally composed of one or more viral proteins, such as, but not limited to those proteins referred to as capsid, coat, shell, surface and/or envelope proteins, or particle-forming polypeptides derived from these proteins. VLPs can form spontaneously upon recombinant expression of the protein in an appropriate expression system. Methods for producing particular VLPs are known in the art and discussed more fully below. The presence of VLPs following recombinant expression of viral proteins can be detected using conventional techniques known in the art, such as by electron microscopy, X-ray crystallography, and the like. See, e.g., Baker et al., *Biophys. J.* (1991) 60:1445-1456; Hagensee et al., *J. Virol.* (1994) 68:4503-4505. For example, VLPs can be isolated by density gradient centrifugation and/or identified by characteristic density banding. Alternatively, cryoelectron microscopy can be performed on vitrified aqueous samples of the VLP preparation in question, and images recorded under appropriate exposure conditions.

By "particle-forming polypeptide" derived from a particular viral protein is meant a full-length or near full-length viral protein, as well as a fragment thereof, or a viral protein with internal deletions, which has the ability to form VLPs under conditions that favor VLP formation. Accordingly, the polypeptide may comprise the full-length sequence, fragments, truncated and partial sequences, as well as analogs and precursor forms of the reference molecule. The term therefore intends deletions, additions and substitutions to the sequence, so long as the polypeptide retains the ability to form a VLP. Thus, the term includes natural variations of the specified polypeptide since variations in coat proteins often occur between viral isolates. The term also includes deletions, additions and substitutions that do not naturally occur in the reference protein, so long as the protein retains the ability to form a VLP.

Preferred substitutions are those which are conservative in nature, i.e., those substitutions that take place within a family of amino acids that are related in their side chains. Specifically, amino acids are generally divided into four families: (1) acidic -- aspartate and glutamate; (2) basic -- lysine, arginine, histidine; (3) non-polar -- alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar -- glycine, asparagine, glutamine, cystine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids.

An "antigen" refers to a molecule containing one or more epitopes (either linear, conformational or both) that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term is used interchangeably with the term "immunogen." Normally, a B-cell epitope will include at least about 5 amino acids but can be as small as 3-4 amino acids. A T-cell epitope, such as a CTL epitope, will include at least about 7-9 amino acids, and a helper T-cell epitope at least about 12-20 amino acids. Normally, an epitope will include between about 7 and 15 amino acids, such as, 9, 10, 12 or 15 amino acids. The term "antigen" denotes both subunit antigens, (i.e., antigens which are separate and discrete from a whole organism with which the antigen is associated in nature), as well as, killed, attenuated or inactivated bacteria, viruses, fungi, parasites or other microbes. Antibodies such as anti-idiotype antibodies, or fragments thereof, and synthetic peptide mimotopes, which can mimic an antigen or antigenic determinant, are also captured under the definition of antigen as used herein. Similarly, an oligonucleotide or polynucleotide which expresses an antigen or antigenic determinant *in vivo*, such as in gene therapy and DNA immunization applications, is also included in the definition of antigen herein.

For purposes of the present invention, antigens can be derived from any of several known viruses, bacteria, parasites and fungi, as described more fully below. The term also intends any of the various tumor antigens. Furthermore, for purposes of the present invention, an "antigen" refers to a protein which includes modifications, such as deletions, additions and substitutions (generally conservative in nature), to the native sequence, so long as the protein maintains the ability to elicit an immunological response, as defined herein. These modifications may be deliberate,

as through site-directed mutagenesis, or may be accidental, such as through mutations of hosts which produce the antigens.

An "immunological response" to an antigen or composition is the development in a subject of a humoral and/or a cellular immune response to an antigen present in the composition of interest. For purposes of the present invention, a "humoral immune response" refers to an immune response mediated by antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells. One important aspect of cellular immunity involves an antigen-specific response by cytolytic T-cells ("CTL"s). CTLs have specificity for peptide antigens that are presented in association with proteins encoded by the major histocompatibility complex (MHC) and expressed on the surfaces of cells. CTLs help induce and promote the destruction of intracellular microbes, or the lysis of cells infected with such microbes. Another aspect of cellular immunity involves an antigen-specific response by helper T-cells. Helper T-cells act to help stimulate the function, and focus the activity of, nonspecific effector cells against cells displaying peptide antigens in association with MHC molecules on their surface. A "cellular immune response" also refers to the production of cytokines, chemokines and other such molecules produced by activated T-cells and/or other white blood cells, including those derived from CD4+ and CD8+ T-cells.

A composition or vaccine that elicits a cellular immune response may serve to sensitize a vertebrate subject by the presentation of antigen in association with MHC molecules at the cell surface. The cell-mediated immune response is directed at, or near, cells presenting antigen at their surface. In addition, antigen-specific T-lymphocytes can be generated to allow for the future protection of an immunized host.

The ability of a particular antigen to stimulate a cell-mediated immunological response may be determined by a number of assays, such as by lymphoproliferation (lymphocyte activation) assays, CTL cytotoxic cell assays, or by assaying for T-lymphocytes specific for the antigen in a sensitized subject. Such assays are well known in the art. See, e.g., Erickson et al., *J. Immunol.* (1993) 151:4189-4199; Doe et al., *Eur. J. Immunol.* (1994) 24:2369-2376. Recent methods of measuring cell-mediated immune response include measurement of intracellular cytokines or cytokine secretion by T-cell populations, or by measurement of epitope specific T-

cells (e.g., by the tetramer technique)(reviewed by McMichael, A.J., and O'Callaghan, C.A., *J. Exp. Med.* 187(9)1367-1371, 1998; Mcheyzer-Williams, M.G., et al, *Immunol. Rev.* 150:5-21, 1996; Lalvani, A., et al, *J. Exp. Med.* 186:859-865, 1997).

Thus, an immunological response as used herein may be one which stimulates  
5 the production of CTLs, and/or the production or activation of helper T- cells. The antigen of interest may also elicit an antibody-mediated immune response. Hence, an immunological response may include one or more of the following effects: the production of antibodies by B-cells; and/or the activation of suppressor T-cells and/or γδ T-cells directed specifically to an antigen or antigens present in the composition or  
10 vaccine of interest. These responses may serve to neutralize infectivity, and/or mediate antibody-complement, or antibody dependent cell cytotoxicity (ADCC) to provide protection to an immunized host. Such responses can be determined using standard immunoassays and neutralization assays, well known in the art.

An "immunogenic composition" is a composition that comprises an antigenic  
15 molecule where administration of the composition to a subject results in the development in the subject of a humoral and/or a cellular immune response to the antigenic molecule of interest. The immunogenic composition can be introduced directly into a recipient subject, such as by injection, inhalation, oral, intranasal and mucosal (e.g., intra-rectally or intra-vaginally) administration.

By "subunit vaccine" is meant a vaccine composition which includes one or  
20 more selected antigens but not all antigens, derived from or homologous to, an antigen from a pathogen of interest such as from a virus, bacterium, parasite or fungus. Such a composition is substantially free of intact pathogen cells or pathogenic particles, or the lysate of such cells or particles. Thus, a "subunit vaccine"  
25 can be prepared from at least partially purified (preferably substantially purified) immunogenic polypeptides from the pathogen, or analogs thereof. The method of obtaining an antigen included in the subunit vaccine can thus include standard purification techniques, recombinant production, or synthetic production.

"Substantially purified" general refers to isolation of a substance (compound,  
30 polynucleotide, protein, polypeptide, polypeptide composition) such that the substance comprises the majority percent of the sample in which it resides. Typically in a sample a substantially purified component comprises 50%, preferably 80%-85%,

more preferably 90-95% of the sample. Techniques for purifying polynucleotides and polypeptides of interest are well-known in the art and include, for example, ion-exchange chromatography, affinity chromatography and sedimentation according to density.

5 A "coding sequence" or a sequence which "encodes" a selected polypeptide, is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide *in vivo* when placed under the control of appropriate regulatory sequences (or "control elements"). The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a  
10 translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from viral, prokaryotic or eucaryotic mRNA, genomic DNA sequences from viral or prokaryotic DNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

15 Typical "control elements", include, but are not limited to, transcription promoters, transcription enhancer elements, transcription termination signals, polyadenylation sequences (located 3' to the translation stop codon), sequences for optimization of initiation of translation (located 5' to the coding sequence), and translation termination sequences.

20 A "nucleic acid" molecule can include, but is not limited to, prokaryotic sequences, eucaryotic mRNA, cDNA from eucaryotic mRNA, genomic DNA sequences from eucaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. The term also captures sequences that include any of the known base analogs of DNA and RNA.

25 "Operably linked" refers to an arrangement of elements wherein the components so described are configured so as to perform their usual function. Thus, a given promoter operably linked to a coding sequence is capable of effecting the expression of the coding sequence when the proper enzymes are present. The promoter need not be contiguous with the coding sequence, so long as it functions to direct the expression thereof. Thus, for example, intervening untranslated yet  
30 transcribed sequences can be present between the promoter sequence and the coding sequence and the promoter sequence can still be considered "operably linked" to the coding sequence.

"Recombinant" as used herein to describe a nucleic acid molecule means a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation: (1) is not associated with all or a portion of the polynucleotide with which it is associated in nature; and/or (2) is linked to a 5 polynucleotide other than that to which it is linked in nature. The term "recombinant" as used with respect to a protein or polypeptide means a polypeptide produced by expression of a recombinant polynucleotide. "Recombinant host cells," "host cells," "cells," "cell lines," "cell cultures," and other such terms denoting prokaryotic microorganisms or eukaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for 10 recombinant vectors or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement to the original parent, due to accidental or deliberate mutation.

15 Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended by this definition, and are covered by the above terms.

Techniques for determining amino acid sequence "similarity" are well known 20 in the art. In general, "similarity" means the exact amino acid to amino acid comparison of two or more polypeptides at the appropriate place, where amino acids are identical or possess similar chemical and/or physical properties such as charge or hydrophobicity. A so-termed "percent similarity" then can be determined between the compared polypeptide sequences. Techniques for determining nucleic acid and amino 25 acid sequence identity also are well known in the art and include determining the nucleotide sequence of the mRNA for that gene (usually via a cDNA intermediate) and determining the amino acid sequence encoded thereby, and comparing this to a second amino acid sequence. In general, "identity" refers to an exact nucleotide to nucleotide or amino acid to amino acid correspondence of two polynucleotides or 30 polypeptide sequences, respectively.

Two or more polynucleotide sequences can be compared by determining their "percent identity." Two or more amino acid sequences likewise can be compared by

determining their "percent identity." The percent identity of two sequences, whether nucleic acid or peptide sequences, is generally described as the number of exact matches between two aligned sequences divided by the length of the shorter sequence and multiplied by 100. An approximate alignment for nucleic acid sequences is

5 provided by the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981). This algorithm can be extended to use with peptide sequences using the scoring matrix developed by Dayhoff, *Atlas of Protein Sequences and Structure*, M.O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA, and normalized by Gribskov, *Nucl.*

10 *Acids Res.* 14(6):6745-6763 (1986). An implementation of this algorithm for nucleic acid and peptide sequences is provided by the Genetics Computer Group (Madison, WI) in their BestFit utility application. The default parameters for this method are described in the Wisconsin Sequence Analysis Package Program Manual, Version 8 (1995) (available from Genetics Computer Group, Madison, WI). Other equally

15 suitable programs for calculating the percent identity or similarity between sequences are generally known in the art.

For example, percent identity of a particular nucleotide sequence to a reference sequence can be determined using the homology algorithm of Smith and Waterman with a default scoring table and a gap penalty of six nucleotide positions. Another

20 method of establishing percent identity in the context of the present invention is to use the MPSRCH package of programs copyrighted by the University of Edinburgh, developed by John F. Collins and Shane S. Sturrok, and distributed by IntelliGenetics, Inc. (Mountain View, CA). From this suite of packages, the Smith-Waterman algorithm can be employed where default parameters are used for the scoring table

25 (for example, gap open penalty of 12, gap extension penalty of one, and a gap of six). From the data generated, the "Match" value reflects "sequence identity." Other suitable programs for calculating the percent identity or similarity between sequences are generally known in the art, such as the alignment program BLAST, which can also be used with default parameters. For example, BLASTN and BLASTP can be used

30 with the following default parameters: genetic code = standard; filter = none; strand = both; cutoff = 60; expect = 10; Matrix = BLOSUM62; Descriptions = 50 sequences; sort by = HIGH SCORE; Databases = non-redundant, GenBank + EMBL + DDBJ +

PDB + GenBank CDS translations + Swiss protein + Spupdate + PIR. Details of these programs can be found at the following internet address:  
<http://www.ncbi.nlm.gov/cgi-bin/BLAST>.

One of skill in the art can readily determine the proper search parameters to  
5 use for a given sequence in the above programs. For example, the search parameters  
may vary based on the size of the sequence in question. Thus, for example, a  
representative embodiment of the present invention would include an isolated  
polynucleotide having X contiguous nucleotides, wherein (i) the X contiguous  
nucleotides have at least about 50% identity to Y contiguous nucleotides derived from  
10 any of the sequences described herein, (ii) X equals Y, and (iii) X is greater than or  
equal to 6 nucleotides and up to 5000 nucleotides, preferably greater than or equal to  
8 nucleotides and up to 5000 nucleotides, more preferably 10-12 nucleotides and up to  
5000 nucleotides, and even more preferably 15-20 nucleotides, up to the number of  
nucleotides present in the full-length sequences described herein (e.g., see the  
15 Sequence Listing and claims), including all integer values falling within the above-  
described ranges.

The synthetic expression cassettes (and purified polynucleotides) of the  
present invention include related polynucleotide sequences having about 80% to  
100%, greater than 80-85%, preferably greater than 90-92%, more preferably greater  
20 than 95%, and most preferably greater than 98% sequence (including all integer  
values falling within these described ranges) identity to the synthetic expression  
cassette sequences disclosed herein (for example, to the claimed sequences or other  
sequences of the present invention) when the sequences of the present invention are  
used as the query sequence.

25 Two nucleic acid fragments are considered to "selectively hybridize" as  
described herein. The degree of sequence identity between two nucleic acid  
molecules affects the efficiency and strength of hybridization events between such  
molecules. A partially identical nucleic acid sequence will at least partially inhibit a  
completely identical sequence from hybridizing to a target molecule. Inhibition of  
30 hybridization of the completely identical sequence can be assessed using  
hybridization assays that are well known in the art (e.g., Southern blot, Northern blot,  
solution hybridization, or the like, see Sambrook, et al., *supra* or Ausubel et al.,

*supra*). Such assays can be conducted using varying degrees of selectivity, for example, using conditions varying from low to high stringency. If conditions of low stringency are employed, the absence of non-specific binding can be assessed using a secondary probe that lacks even a partial degree of sequence identity (for example, a 5 probe having less than about 30% sequence identity with the target molecule), such that, in the absence of non-specific binding events, the secondary probe will not hybridize to the target.

When utilizing a hybridization-based detection system, a nucleic acid probe is chosen that is complementary to a target nucleic acid sequence, and then by selection 10 of appropriate conditions the probe and the target sequence "selectively hybridize," or bind, to each other to form a hybrid molecule. A nucleic acid molecule that is capable of hybridizing selectively to a target sequence under "moderately stringent" typically hybridizes under conditions that allow detection of a target nucleic acid sequence of at least about 10-14 nucleotides in length having at least approximately 70% sequence 15 identity with the sequence of the selected nucleic acid probe. Stringent hybridization conditions typically allow detection of target nucleic acid sequences of at least about 10-14 nucleotides in length having a sequence identity of greater than about 90-95% with the sequence of the selected nucleic acid probe. Hybridization conditions useful for probe/target hybridization where the probe and target have a specific degree of 20 sequence identity, can be determined as is known in the art (see, for example, Nucleic Acid Hybridization: A Practical Approach, editors B.D. Hames and S.J. Higgins, (1985) Oxford; Washington, DC; IRL Press).

With respect to stringency conditions for hybridization, it is well known in the art that numerous equivalent conditions can be employed to establish a particular 25 stringency by varying, for example, the following factors: the length and nature of probe and target sequences, base composition of the various sequences, concentrations of salts and other hybridization solution components, the presence or absence of blocking agents in the hybridization solutions (e.g., formamide, dextran sulfate, and polyethylene glycol), hybridization reaction temperature and time 30 parameters, as well as, varying wash conditions. The selection of a particular set of hybridization conditions is selected following standard methods in the art (see, for example, Sambrook, et al., *supra* or Ausubel et al., *supra*).

A first polynucleotide is "derived from" second polynucleotide if it has the same or substantially the same basepair sequence as a region of the second polynucleotide, its cDNA, complements thereof, or if it displays sequence identity as described above.

- 5 A first polypeptide is "derived from" a second polypeptide if it is (i) encoded by a first polynucleotide derived from a second polynucleotide, or (ii) displays sequence identity to the second polypeptides as described above.

- Generally, a viral polypeptide is "derived from" a particular polypeptide of a virus (viral polypeptide) if it is (i) encoded by an open reading frame of a  
10 polynucleotide of that virus (viral polynucleotide), or (ii) displays sequence identity to polypeptides of that virus as described above.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence of at least 3 to 5 amino acids, more preferably at least 8 to 10 amino  
15 acids, and even more preferably at least 15 to 20 amino acids from a polypeptide encoded by the nucleic acid sequence. Also encompassed are polypeptide sequences which are immunologically identifiable with a polypeptide encoded by the sequence.

"Purified polynucleotide" refers to a polynucleotide of interest or fragment thereof which is essentially free, e.g., contains less than about 50%, preferably less  
20 than about 70%, and more preferably less than about 90%, of the protein with which the polynucleotide is naturally associated. Techniques for purifying polynucleotides of interest are well-known in the art and include, for example, disruption of the cell containing the polynucleotide with a chaotropic agent and separation of the polynucleotide(s) and proteins by ion-exchange chromatography, affinity  
25 chromatography and sedimentation according to density.

By "nucleic acid immunization" is meant the introduction of a nucleic acid molecule encoding one or more selected antigens into a host cell, for the *in vivo* expression of an antigen, antigens, an epitope, or epitopes. The nucleic acid molecule can be introduced directly into a recipient subject, such as by injection, inhalation,  
30 oral, intranasal and mucosal administration, or the like, or can be introduced *ex vivo*, into cells which have been removed from the host. In the latter case, the transformed

cells are reintroduced into the subject where an immune response can be mounted against the antigen encoded by the nucleic acid molecule.

- "Gene transfer" or "gene delivery" refers to methods or systems for reliably inserting DNA of interest into a host cell. Such methods can result in transient expression of non-integrated transferred DNA, extrachromosomal replication and expression of transferred replicons (e.g., episomes), or integration of transferred genetic material into the genomic DNA of host cells. Gene delivery expression vectors include, but are not limited to, vectors derived from alphaviruses, pox viruses and vaccinia viruses. When used for immunization, such gene delivery expression vectors may be referred to as vaccines or vaccine vectors.

"T lymphocytes" or "T cells" are non-antibody producing lymphocytes that constitute a part of the cell-mediated arm of the immune system. T cells arise from immature lymphocytes that migrate from the bone marrow to the thymus, where they undergo a maturation process under the direction of thymic hormones. Here, the mature lymphocytes rapidly divide increasing to very large numbers. The maturing T cells become immunocompetent based on their ability to recognize and bind a specific antigen. Activation of immunocompetent T cells is triggered when an antigen binds to the lymphocyte's surface receptors.

- The term "transfection" is used to refer to the uptake of foreign DNA by a cell. A cell has been "transfected" when exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are generally known in the art. See, e.g., Graham et al. (1973) *Virology*, 52:456, Sambrook et al. (1989) *Molecular Cloning, a laboratory manual*, Cold Spring Harbor Laboratories, New York, Davis et al. (1986) *Basic Methods in Molecular Biology*, Elsevier, and Chu et al. (1981) *Gene* 13:197. Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells. The term refers to both stable and transient uptake of the genetic material, and includes uptake of peptide- or antibody-linked DNAs.

A "vector" is capable of transferring gene sequences to target cells (e.g., viral vectors, non-viral vectors, particulate carriers, and liposomes). Typically, "vector construct," "expression vector," and "gene transfer vector," mean any nucleic acid construct capable of directing the expression of a gene of interest and which can

transfer gene sequences to target cells. Thus, the term includes cloning and expression vehicles, as well as viral vectors.

Transfer of a "suicide gene" (e.g., a drug-susceptibility gene) to a target cell renders the cell sensitive to compounds or compositions that are relatively nontoxic to normal cells. Moolten, F.L. (1994) *Cancer Gene Ther.* 1:279-287. Examples of suicide genes are thymidine kinase of herpes simplex virus (HSV-tk), cytochrome P450 (Manome et al. (1996) *Gene Therapy* 3:513-520), human deoxycytidine kinase (Manome et al. (1996) *Nature Medicine* 2(5):567-573) and the bacterial enzyme cytosine deaminase (Dong et al. (1996) *Human Gene Therapy* 7:713-720). Cells which express these genes are rendered sensitive to the effects of the relatively nontoxic prodrugs ganciclovir (HSV-tk), cyclophosphamide (cytochrome P450 2B1), cytosine arabinoside (human deoxycytidine kinase) or 5-fluorocytosine (bacterial cytosine deaminase). Culver et al. (1992) *Science* 256:1550-1552, Huber et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:8302-8306.

A "selectable marker" or "reporter marker" refers to a nucleotide sequence included in a gene transfer vector that has no therapeutic activity, but rather is included to allow for simpler preparation, manufacturing, characterization or testing of the gene transfer vector.

A "specific binding agent" refers to a member of a specific binding pair of molecules wherein one of the molecules specifically binds to the second molecule through chemical and/or physical means. One example of a specific binding agent is an antibody directed against a selected antigen.

By "subject" is meant any member of the subphylum chordata, including, without limitation, humans and other primates, including non-human primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats and guinea pigs; birds, including domestic, wild and game birds such as chickens, turkeys and other gallinaceous birds, ducks, geese, and the like. The term does not denote a particular age. Thus, both adult and newborn individuals are intended to be covered. The system described above is intended for use in any of the above vertebrate species, since the immune systems of all of these vertebrates operate similarly.

By "pharmaceutically acceptable" or "pharmacologically acceptable" is meant a material which is not biologically or otherwise undesirable, i.e., the material may be administered to an individual in a formulation or composition without causing any undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained.

5 By "physiological pH" or a "pH in the physiological range" is meant a pH in the range of approximately 7.2 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

As used herein, "treatment" refers to any of (I) the prevention of infection or 10 reinfection, as in a traditional vaccine, (ii) the reduction or elimination of symptoms, and (iii) the substantial or complete elimination of the pathogen in question.

Treatment may be effected prophylactically (prior to infection) or therapeutically (following infection).

"Lentiviral vector", and "recombinant lentiviral vector" refer to a nucleic acid 15 construct which carries, and within certain embodiments, is capable of directing the expression of a nucleic acid molecule of interest. The lentiviral vector include at least one transcriptional promoter/enhancer or locus defining element(s), or other elements which control gene expression by other means such as alternate splicing, nuclear RNA export, post-translational modification of messenger, or post-transcriptional 20 modification of protein. Such vector constructs must also include a packaging signal, long terminal repeats (LTRS) or portion thereof, and positive and negative strand primer binding sites appropriate to the retrovirus used (if these are not already present in the retroviral vector). Optionally, the recombinant lentiviral vector may also include a signal which directs polyadenylation, selectable markers such as Neo, TK, 25 hygromycin, phleomycin, histidinol, or DHFR, as well as one or more restriction sites and a translation termination sequence. By way of example, such vectors typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second strand DNA synthesis, and a 3'LTR or a portion thereof.

"Lentiviral vector particle" as utilized within the present invention refers to a 30 lentivirus which carries at least one gene of interest. The retrovirus may also contain a selectable marker. The recombinant lentivirus is capable of reverse transcribing its genetic material (RNA) into DNA and incorporating this genetic material into a host

cell's DNA upon infection. Lentiviral vector particles may have a lentiviral envelope, a non-lentiviral envelope (e.g., an amphi or VSV-G envelope), or a chimeric envelope.

"Nucleic acid expression vector" or "Expression cassette" refers to an assembly which is capable of directing the expression of a sequence or gene of interest. The nucleic acid expression vector includes a promoter which is operably linked to the sequences or gene(s) of interest. Other control elements may be present as well. Expression cassettes described herein may be contained within a plasmid construct. In addition to the components of the expression cassette, the plasmid construct may also include a bacterial origin of replication, one or more selectable markers, a signal which allows the plasmid construct to exist as single-stranded DNA (e.g., a M13 origin of replication), a multiple cloning site, and a "mammalian" origin of replication (e.g., a SV40 or adenovirus origin of replication).

"Packaging cell" refers to a cell which contains those elements necessary for production of infectious recombinant retrovirus which are lacking in a recombinant retroviral vector. Typically, such packaging cells contain one or more expression cassettes which are capable of expressing proteins which encode *Gag*, *pol* and *env* proteins.

"Producer cell" or "vector producing cell" refers to a cell which contains all elements necessary for production of recombinant retroviral vector particles.

## 2. MODES OF CARRYING OUT THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particular formulations or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting.

Although a number of methods and materials similar or equivalent to those described herein can be used in the practice of the present invention, the preferred materials and methods are described herein.

## 2.1 SYNTHETIC EXPRESSION CASSETTES

### 2.1.1 MODIFICATION OF HIV-1-TYPE C GAG AND ENV NUCLEIC ACID CODING SEQUENCES

One aspect of the present invention is the generation of HIV-1 type C Gag and Env protein coding sequences, and related sequences, having improved expression relative to the corresponding wild-type sequences.

#### 2.1.1.1 MODIFICATION OF GAG NUCLEIC ACID CODING SEQUENCES

An exemplary embodiment of the present invention is illustrated herein by modifying the Gag protein wild-type sequences obtained from the AF110965 and AF110967 strains of HIV-1, subtype C. (see, for example, Korber et al. (1998) *Human Retroviruses and Aids*, Los Alamos, New Mexico: Los Alamos National Laboratory; Novitsky et al. (1999) *J. Virol.* 73(5):4427-4432, for molecular cloning of various subtype C clones from Botswana). Gag sequence obtained from other Type C HIV-1 variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include, but are not limited to, Gag protein encoding sequences obtained from the isolates of HIV-1 Type C, for example as described in Novitsky et al., (1999), *supra*; Myers et al., *infra*; Virology, 3rd Edition (W.K. Joklik ed. 1988); *Fundamental Virology*, 2nd Edition (B.N. Fields and D.M. Knipe, eds. 1991); *Virology*, 3rd Edition (Fields, BN, DM Knipe, PM Howley, Editors, 1996, Lippincott-Raven, Philadelphia, PA and on the World Wide Web (Internet), for example at <http://hiv-web.lan1.gov/cgi-bin/hivDB3/public/wdb/ssampublic> and <http://hiv-web.lan1.gov>.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes (Example 1). The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The Gag coding sequences were modified to be comparable to codon usage found in highly expressed human genes.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag coding sequences. The RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating effects of the INS. To overcome the post-transcriptional activating mechanisms of RRE and Rev, the instability elements can be inactivated by introducing multiple point mutations that do not alter the reading frame of the encoded proteins. Subtype C Gag-encoding sequences having inactivated RRE sites are shown in Figures 1 (SEQ ID NO:3), 2 (SEQ ID NO:4), 5 (SEQ ID NO:20) and 6 (SEQ ID NO:26).

10 Modification of the Gag polypeptide coding sequences results in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells). Further, expression of the sequences results in production of virus-like particles (VLPs) by these cell lines (see below).

15

#### 2.1.1.2 MODIFICATION OF *Env* NUCLEIC ACID CODING SEQUENCES

Similarly, the present invention also includes modified Env proteins. Wild-type Env sequences are obtained from the AF110968 and AF110975 strains of HIV-1, type C. (see, for example, Novitsky et al. (1999) *J. Virol.* 73(5):4427-4432, for 20 molecular cloning of various subtype C clones from Botswana). Env sequence obtained from other Type C HIV-1 variants may be manipulated in similar fashion following the teachings of the present specification. Such other variants include, but are not limited to, Env protein encoding sequences obtained from the isolates of HIV-1 Type C, described above.

25 The codon usage pattern for Env was modified as described above for Gag so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed human genes. Experiments can be performed in support of the present invention to show that the synthetic Env sequences were capable of higher level of protein production relative to the native Env sequences.

30 Modification of the Env polypeptide coding sequences results in improved expression relative to the wild-type coding sequences in a number of mammalian cell lines (as well as other types of cell lines, including, but not limited to, insect cells).

Similar Env polypeptide coding sequences can be obtained, optimized and tested for improved expression from a variety of isolates, including those described above for Gag.

5           **2.1.2 FURTHER MODIFICATION OF SEQUENCES INCLUDING HIV-1 *GAG* NUCLEIC ACID CODING SEQUENCES**

Experiments can be performed to show that similar modifications of HIV-1 Gag-protease and Gag-polymerase sequences also result in improved expression of the polyproteins, as well as, the production of VLPs formed by polypeptides produced 10 from such modified coding sequences.

For the Gag-protease sequence, the changes in codon usage are typically restricted to the regions up to the -1 frameshift and starting again at the end of the Gag reading frame; however, regions within the frameshift translation region can be modified as well. Further, inhibitory (or instability) elements (INS) located within the 15 coding sequences of the Gag-protease polypeptide coding sequence can be altered as well.

For the Gag-polymerase sequence, the changes in codon usage can be similar to those for the Gag-protease sequence.

In addition to polyproteins containing HIV-related sequences, the Gag 20 encoding sequences of the present invention can be fused to other polypeptides (creating chimeric polypeptides) for which an immunogenic response is desired.

Further sequences useful in the practice of the present invention include, but are not limited to, sequences encoding further viral epitopes/antigens {including but 25 not limited to, HCV antigens (e.g., E1, E2; Houghton, M., et al., U.S. Patent No. 5,714,596, issued February 3, 1998; Houghton, M., et al., U.S. Patent No. 5,712,088, issued January 27, 1998; Houghton, M., et al., U.S. Patent No. 5,683,864, issued November 4, 1997; Weiner, A.J., et al., U.S. Patent No. 5,728,520, issued March 17, 1998; Weiner, A.J., et al., U.S. Patent No. 5,766,845, issued June 16, 1998; Weiner, A.J., et al., U.S. Patent No. 5,670,152, issued September 23, 1997), HIV antigens 30 (e.g., derived from *tat*, *rev*, *nef* and/or *env*); and sequences encoding tumor antigens/epitopes. Additional sequences are described below. Also, variations on the

orientation of the Gag and other coding sequences, relative to each other, are described below.

- Gag, Gag-protease, and/or Gag-polymerase polypeptide coding sequences can be obtained from Type C HIV isolates, see, e.g., Myers et al. Los Alamos Database, 5 Los Alamos National Laboratory, Los Alamos, New Mexico (1992); Myers et al., *Human Retroviruses and Aids, 1997*, Los Alamos, New Mexico: Los Alamos National Laboratory. Synthetic expression cassettes can be generated using such coding sequences as starting material by following the teachings of the present specification (e.g., see Example 1).
- 10 Further, the synthetic expression cassettes of the present invention include related Gag polypeptide coding sequences having greater than 85%, preferably greater than 90%, more preferably greater than 95%, and most preferably greater than 98% sequence identity to the synthetic expression cassette sequences disclosed herein (for example, Figures 1, 2, 5 and 6 (SEQ ID NOs:3, 4, 20 and 21)).
- 15 The present invention also includes related Env polypeptide coding sequences having greater than 85%, preferably greater than 90%, more preferably greater than 95%, and most preferably greater than 98% sequence identity to the sequences disclosed herein (for example, Figures 3 and 4, SEQ ID NOs:5-17). Various coding regions are indicated in Figures 3 and 4, for example in Figure 3 (AF110968), 20 nucleotides 1-81 (SEQ ID NO:18) encode a signal peptide, nucleotides 82-1512 (SEQ ID NO:6) encode a gp120 polypeptide, nucleotides 1513 to 2547 (SEQ ID NO:10) encode a gp41 polypeptide, nucleotides 82-2025 (SEQ ID NO:7) encode a gp140 polypeptide and nucleotides 82-2547 (SEQ ID NO:8) encode a gp160 polypeptide.

25 **2.1.3 EXPRESSION OF SYNTHETIC SEQUENCES ENCODING HIV-1 GAG OR ENV AND RELATED POLYPEPTIDES**

Synthetic Gag- and Env-encoding sequences (expression cassettes) of the present invention can be cloned into a number of different expression vectors to evaluate levels of expression and, in the case of Gag, production of VLPs. The 30 synthetic DNA fragments for Env and Gag can be cloned into eucaryotic expression vectors, including, a transient expression vector, CMV-promoter-based mammalian

vectors, and a shuttle vector for use in baculovirus expression systems.

Corresponding wild-type sequences can also be cloned into the same vectors.

These vectors can then be transfected into a several different cell types, including a variety of mammalian cell lines,(293, RD, COS-7, and CHO, cell lines 5 available, for example, from the A.T.C.C.). The cell lines are then cultured under appropriate conditions and the levels of p24 (Gag) or, gp160 or gp120 (Env) expression in supernatants can be evaluated (Example 2). Env polypeptides include, but are not limited to, for example, native gp160, oligomeric gp140, monomeric gp120 as well as modified sequences of these polypeptides. The results of these 10 assays demonstrate that expression of synthetic Env, Gag and Gag-protease encoding sequences are significantly higher than corresponding wild-type sequences.

Further, Western Blot analysis can be used to show that cells containing the synthetic Gag or Env expression cassette produce the expected protein at higher per-cell concentrations than cells containing the native expression cassette. The Gag and 15 Env proteins can be seen in both cell lysates and supernatants. The levels of production are significantly higher in cell supernatants for cells transfected with the synthetic expression cassettes of the present invention.

Fractionation of the supernatants from mammalian cells transfected with the synthetic Gag or Env expression cassette can be used to show that the cassettes 20 provide superior production of both Gag and Env proteins and, in the case of Gag, VLPs, relative to the wild-type sequences.

Efficient expression of these Gag- and/or Env-containing polypeptides in mammalian cell lines provides the following benefits: the polypeptides are free of baculovirus contaminants; production by established methods approved by the FDA; 25 increased purity; greater yields (relative to native coding sequences); and a novel method of producing the Gag- and/or Env-containing polypeptides in CHO cells which is not feasible in the absence of the increased expression obtained using the constructs of the present invention. Exemplary Mammalian cell lines include, but are not limited to, BHK, VERO, HT1080, 293, 293T, RD, COS-7, CHO, Jurkat, HUT, 30 SUPT, C8166, MOLT4/clone8, MT-2, MT-4, H9, PM1, CEM, and CEMX174, such cell lines are available, for example, from the A.T.C.C.).

A synthetic Gag expression cassette of the present invention will also exhibit high levels of expression and VLP production when transfected into insect cells. Synthetic Env expression cassettes also demonstrate high levels of expression in insect cells. Further, in addition to a higher total protein yield, the final product from the synthetic polypeptides consistently contains lower amounts of contaminating baculovirus proteins than the final product from the native Gag or Env.

Further, synthetic Gag and Env expression cassettes of the present invention can also be introduced into yeast vectors which, in turn, can be transformed into and efficiently expressed by yeast cells (*Saccharomyces cerevisiae*; using vectors as described in Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998).

In addition to the mammalian and insect vectors, the synthetic expression cassettes of the present invention can be incorporated into a variety of expression vectors using selected expression control elements. Appropriate vectors and control elements for any given cell type can be selected by one having ordinary skill in the art in view of the teachings of the present specification and information known in the art about expression vectors.

For example, a synthetic Gag or Env expression cassette can be inserted into a vector which includes control elements operably linked to the desired coding sequence, which allow for the expression of the gene in a selected cell-type. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter (a CMV promoter can include intron A), RSV, HIV-Ltr, the mouse mammary tumor virus LTR promoter (MMLV-ltr), the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression. Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook, et al., *supra*, as well as a bovine growth hormone terminator sequence. Introns, containing splice

donor and acceptor sites, may also be designed into the constructs for use with the present invention (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as elements included in the CMV intron A sequence (Chapman et al., *Nuc. Acids Res.* (1991) 19:3979-3986).

The desired synthetic Gag or Env polypeptide encoding sequences can be cloned into any number of commercially available vectors to generate expression of the polypeptide in an appropriate host system. These systems include, but are not limited to, the following: baculovirus expression {Reilly, P.R., et al., BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL (1992); Beames, et al., *Biotechniques* 11:378 (1991); Pharmingen; Clontech, Palo Alto, CA)}, vaccinia expression {Earl, P. L., et al., "Expression of proteins in mammalian cells using vaccinia" In *Current Protocols in Molecular Biology* (F. M. Ausubel, et al. Eds.), Greene Publishing Associates & Wiley Interscience, New York (1991); Moss, B., et al., U.S. Patent Number 5,135,855, issued 4 August 1992}, expression in bacteria {Ausubel, F.M., et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley and Sons, Inc., Media PA; Clontech}, expression in yeast {Rosenberg, S. and Tekamp-Olson, P., U.S. Patent No. RE35,749, issued, March 17, 1998; Shuster, J.R., U.S. Patent No. 5,629,203, issued May 13, 1997; Gellissen, G., et al., *Antonie Van Leeuwenhoek*, 62(1-2):79-93 (1992); Romanos, M.A., et al., *Yeast* 8(6):423-488 (1992); Goeddel, D.V., *Methods in Enzymology* 185 (1990); Guthrie, C., and G.R. Fink, *Methods in Enzymology* 194 (1991)}, expression in mammalian cells {Clontech; Gibco-BRL, Ground Island, NY; e.g., Chinese hamster ovary (CHO) cell lines (Haynes, J., et al., *Nuc. Acid. Res.* 11:687-706 (1983); 1983, Lau, Y.F., et al., *Mol. Cell. Biol.* 4:1469-1475 (1984); Kaufman, R. J., "Selection and coamplification of heterologous genes in mammalian cells," in *Methods in Enzymology*, vol. 185, pp537-566. Academic Press, Inc., San Diego CA (1991)}, and expression in plant cells

{plant cloning vectors, Clontech Laboratories, Inc., Palo Alto, CA, and Pharmacia LKB Biotechnology, Inc., Piscataway, NJ; Hood, E., *et al.*, *J. Bacteriol.* **168**:1291-1301 (1986); Nagel, R., *et al.*, *FEMS Microbiol. Lett.* **67**:325 (1990); An, *et al.*, "Binary Vectors", and others in *Plant Molecular Biology Manual A3*:1-19 (1988);  
5 Miki, B.L.A., *et al.*, pp.249-265, and others in *Plant DNA Infectious Agents* (Hohn, T., *et al.*, eds.) Springer-Verlag, Wien, Austria, (1987); *Plant Molecular Biology: Essential Techniques*, P.G. Jones and J.M. Sutton, New York, J. Wiley, 1997; Miglani, Gurbachan *Dictionary of Plant Genetics and Molecular Biology*, New York, Food Products Press, 1998; Henry, R. J., *Practical Applications of Plant Molecular Biology*, New York, Chapman & Hall, 1997}.

Also included in the invention is an expression vector, containing coding sequences and expression control elements which allow expression of the coding regions in a suitable host. The control elements generally include a promoter, translation initiation codon, and translation and transcription termination sequences, 15 and an insertion site for introducing the insert into the vector. Translational control elements have been reviewed by M. Kozak (e.g., Kozak, M., *Mamm. Genome* **7**(8):563-574, 1996; Kozak, M., *Biochimie* **76**(9):815-821, 1994; Kozak, M., *J Cell Biol* **108**(2):229-241, 1989; Kozak, M., and Shatkin, A.J., *Methods Enzymol* **60**:360-375, 1979).

20 Expression in yeast systems has the advantage of commercial production. Recombinant protein production by vaccinia and CHO cell line have the advantage of being mammalian expression systems. Further, vaccinia virus expression has several advantages including the following: (i) its wide host range; (ii) faithful post-transcriptional modification, processing, folding, transport, secretion, and assembly of 25 recombinant proteins; (iii) high level expression of relatively soluble recombinant proteins; and (iv) a large capacity to accommodate foreign DNA.

The recombinantly expressed polypeptides from synthetic Gag- and/or Env-encoding expression cassettes are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt 30 fractionation, ion exchange chromatography, gel filtration, size-exclusion chromatography, size-fractionation, and affinity chromatography. Immunoaffinity

chromatography can be employed using antibodies generated based on, for example, Gag or Env antigens.

Advantages of expressing the Gag- and/or Env-containing proteins of the present invention using mammalian cells include, but are not limited to, the following: well-established protocols for scale-up production; the ability to produce VLPs; cell lines are suitable to meet good manufacturing process (GMP) standards; culture conditions for mammalian cells are known in the art.

Various forms of the different embodiments of the invention, described herein, may be combined.

10

## **2.2 PRODUCTION OF VIRUS-LIKE PARTICLES AND USE OF THE CONSTRUCTS OF THE PRESENT INVENTION TO CREATE PACKAGING CELL LINES.**

The group-specific antigens (Gag) of human immunodeficiency virus type-1 (HIV-1) self-assemble into noninfectious virus-like particles (VLP) that are released from various eucaryotic cells by budding (reviewed by Freed, E.O., *Virology* 251:1-15, 1998). The synthetic expression cassettes of the present invention provide efficient means for the production of HIV-Gag virus-like particles (VLPs) using a variety of different cell types, including, but not limited to, mammalian cells.

20 Viral particles can be used as a matrix for the proper presentation of an antigen entrapped or associated therewith to the immune system of the host.

### **2.2.1 VLP PRODUCTION USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION**

25 Experiments can be performed in support of the present invention to demonstrate that the synthetic expression cassettes of the present invention provide superior production of both Gag proteins and VLPs, relative to native Gag coding sequences. Further, electron microscopic evaluation of VLP production can show that free and budding immature virus particles of the expected size are produced by cells 30 containing the synthetic expression cassettes.

Using the synthetic expression cassettes of the present invention, rather than native Gag coding sequences, for the production of virus-like particles provide several

advantages. First, VLPs can be produced in enhanced quantity making isolation and purification of the VLPs easier. Second, VLPs can be produced in a variety of cell types using the synthetic expression cassettes, in particular, mammalian cell lines can be used for VLP production, for example, CHO cells. Production using CHO cells

5 provides (i) VLP formation; (ii) correct myristylation and budding; (iii) absence of non-mammalian cell contaminants (e.g., insect viruses and/or cells); and (iv) ease of purification. The synthetic expression cassettes of the present invention are also useful for enhanced expression in cell-types other than mammalian cell lines. For example, infection of insect cells with baculovirus vectors encoding the synthetic expression

10 cassettes results in higher levels of total Gag protein yield and higher levels of VLP production (relative to wild-type coding sequences). Further, the final product from insect cells infected with the baculovirus-Gag synthetic expression cassettes consistently contains lower amounts of contaminating insect proteins than the final product when wild-type coding sequences are used.

15 VLPs can spontaneously form when the particle-forming polypeptide of interest is recombinantly expressed in an appropriate host cell. Thus, the VLPs produced using the synthetic expression cassettes of the present invention are conveniently prepared using recombinant techniques. As discussed below, the Gag polypeptide encoding synthetic expression cassettes of the present invention can

20 include other polypeptide coding sequences of interest (for example, HIV protease, HIV polymerase, HCV core; Env; synthetic Env; see, Example 1). Expression of such synthetic expression cassettes yields VLPs comprising the Gag polypeptide, as well as, the polypeptide of interest.

Once coding sequences for the desired particle-forming polypeptides have

25 been isolated or synthesized, they can be cloned into any suitable vector or replicon for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. See, generally, Sambrook et al, *supra*. The vector is then used to transform an appropriate host cell. Suitable recombinant expression systems include, but are not limited to, bacterial,

30 mammalian, baculovirus/insect, vaccinia, Semliki Forest virus (SFV), Alphaviruses (such as, Sindbis, Venezuelan Equine Encephalitis (VEE)), mammalian, yeast and

Xenopus expression systems, well known in the art. Particularly preferred expression systems are mammalian cell lines, vaccinia, Sindbis, insect and yeast systems.

For example, a number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (A.T.C.C.), such as, but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), as well as others. Similarly, bacterial hosts such as *E. coli*, *Bacillus subtilis*, and *Streptococcus spp.*, will find use with the present expression constructs. Yeast hosts useful in the present invention include *inter alia*, *Saccharomyces cerevisiae*, *Candida albicans*, *Candida maltosa*, *Hansenula polymorpha*, *Kluyveromyces fragilis*, *Kluyveromyces lactis*, *Pichia guillermondii*, *Pichia pastoris*, *Schizosaccharomyces pombe* and *Yarrowia lipolytica*. Insect cells for use with baculovirus expression vectors include, *inter alia*, *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni*. See, e.g., Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987).

Viral vectors can be used for the production of particles in eucaryotic cells, such as those derived from the pox family of viruses, including vaccinia virus and avian poxvirus. Additionally, a vaccinia based infection/transfection system, as described in Tomei et al., *J. Virol.* (1993) 67:4017-4026 and Selby et al., *J. Gen. Virol.* (1993) 74:1103-1113, will also find use with the present invention. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7 promoters. Following infection, cells are transfected with the DNA of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. Alternately, T7 can be added as a purified protein or enzyme as in the "Progenitor" system (Studier and Moffatt, *J. Mol. Biol.* (1986) 189:113-130). The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its translation product(s).

Depending on the expression system and host selected, the VLPS are produced by growing host cells transformed by an expression vector under conditions whereby

the particle-forming polypeptide is expressed and VLPs can be formed. The selection of the appropriate growth conditions is within the skill of the art. If the VLPs are formed intracellularly, the cells are then disrupted, using chemical, physical or mechanical means, which lyse the cells yet keep the VLPs substantially intact. Such 5 methods are known to those of skill in the art and are described in, e.g., *Protein Purification Applications: A Practical Approach*, (E.L.V. Harris and S. Angal, Eds., 1990).

The particles are then isolated (or substantially purified) using methods that preserve the integrity thereof, such as, by gradient centrifugation, e.g., cesium 10 chloride (CsCl) sucrose gradients, pelleting and the like (see, e.g., Kimbauer et al. *J. Virol.* (1993) 67:6929-6936), as well as standard purification techniques including, e.g., ion exchange and gel filtration chromatography.

VLPs produced by cells containing the synthetic expression cassettes of the present invention can be used to elicit an immune response when administered to a 15 subject. One advantage of the present invention is that VLPs can be produced by mammalian cells carrying the synthetic expression cassettes at levels previously not possible. As discussed above, the VLPs can comprise a variety of antigens in addition to the Gag polypeptide (e.g., Gag-protease, Gag-polymerase, Env, synthetic Env, etc.). Purified VLPs, produced using the synthetic expression cassettes of the 20 present invention, can be administered to a vertebrate subject, usually in the form of vaccine compositions. Combination vaccines may also be used, where such vaccines contain, for example, an adjuvant subunit protein (e.g., Env). Administration can take place using the VLPs formulated alone or formulated with other antigens. Further, the VLPs can be administered prior to, concurrent with, or subsequent to, delivery of 25 the synthetic expression cassettes for DNA immunization (see below) and/or delivery of other vaccines. Also, the site of VLP administration may be the same or different as other vaccine compositions that are being administered. Gene delivery can be accomplished by a number of methods including, but are not limited to, immunization with DNA, alphavirus vectors, pox virus vectors, and vaccinia virus vectors.

30 VLP immune-stimulating (or vaccine) compositions can include various excipients, adjuvants, carriers, auxiliary substances, modulating agents, and the like. The immune stimulating compositions will include an amount of the VLP/antigen

sufficient to mount an immunological response. An appropriate effective amount can be determined by one of skill in the art. Such an amount will fall in a relatively broad range that can be determined through routine trials and will generally be an amount on the order of about 0.1  $\mu$ g to about 1000  $\mu$ g, more preferably about 1  $\mu$ g to about 300  $\mu$ g, of VLP/antigen.

A carrier is optionally present which is a molecule that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen may be conjugated to a bacterial toxoid, such as toxoid from diphtheria, tetanus, cholera, etc., as well as toxins derived from *E. coli*.

Adjuvants may also be used to enhance the effectiveness of the compositions. Such adjuvants include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc.; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (International Publication No. WO 90/14837), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi<sup>TM</sup> adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene,

0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphoryl lipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox<sup>TM</sup>); (3) saponin adjuvants, such as Stimulon<sup>TM</sup> (Cambridge Bioscience, Worcester, MA) may be used or particle 5 generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freunds Adjuvant (CFA) and Incomplete Freunds Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (6) oligonucleotides or polymeric molecules encoding immunostimulatory CpG motifs (Davis, H.L., et al., *J. Immunology* 10 160:870-876, 1998; Sato, Y. et al., *Science* 273:352-354, 1996) or complexes of antigens/oligonucleotides {Polymeric molecules include double and single stranded RNA and DNA, and backbone modifications thereof, for example, methylphosphonate linkages; or (7) detoxified mutants of a bacterial ADP-ribosylating toxin such as a cholera toxin (CT), a pertussis toxin (PT), or an *E. coli* 15 heat-labile toxin (LT), particularly LT-K63 (where lysine is substituted for the wild-type amino acid at position 63) LT-R72 (where arginine is substituted for the wild-type amino acid at position 72), CT-S109 (where serine is substituted for the wild-type amino acid at position 109), and PT-K9/G129 (where lysine is substituted for the wild-type amino acid at position 9 and glycine substituted at position 129) (see, e.g., 20 International Publication Nos. W093/13202 and W092/19265); and (8) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Further, such polymeric molecules include alternative polymer backbone structures such as, but not limited to, polyvinyl backbones (Pitha, *Biochem Biophys Acta*, 204:39, 1970a; Pitha, *Biopolymers*, 9:965, 1970b), and morpholino 25 backbones (Summerton, J., et al., U.S. Patent No. 5,142,047, issued 08/25/92; Summerton, J., et al., U.S. Patent No. 5,185,444 issued 02/09/93). A variety of other charged and uncharged polynucleotide analogs have been reported. Numerous backbone modifications are known in the art, including, but not limited to, uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, and 30 carbamates) and charged linkages (e.g., phosphorothioates and phosphorodithioates).}; and (7) other substances that act as immunostimulating agents

to enhance the effectiveness of the VLP immune-stimulating (or vaccine) composition. Alum, CpG oligonucleotides, and MF59 are preferred.

Muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isogluatme (nor-MDP), N-acetylmuramyl-L-alanyl-D-isogluatminyl-L-alanine-2-(l'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), etc.

Dosage treatment with the VLP composition may be a single dose schedule or a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at 10 subsequent time intervals, chosen to maintain and/or reinforce the immune response, for example at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the subject and be dependent on the judgment of the practitioner.

If prevention of disease is desired, the antigen carrying VLPs are generally 15 administered prior to primary infection with the pathogen of interest. If treatment is desired, e.g., the reduction of symptoms or recurrences, the VLP compositions are generally administered subsequent to primary infection.

### 2.2.2 USING THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT 20 INVENTION TO CREATE PACKAGING CELL LINES

A number of viral based systems have been developed for use as gene transfer vectors for mammalian host cells. For example, retroviruses (in particular, lentiviral vectors) provide a convenient platform for gene delivery systems. A coding sequence of interest (for example, a sequence useful for gene therapy applications) can be 25 inserted into a gene delivery vector and packaged in retroviral particles using techniques known in the art. Recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems have been described, including, for example, the following: (U.S. Patent No. 5,219,740; Miller et al. (1989) *BioTechniques* 7:980; Miller, A.D. (1990) *Human Gene Therapy* 1:5; 30 Scarpa et al. (1991) *Virology* 180:849; Burns et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:8033; Boris-Lawrie et al. (1993) *Cur. Opin. Genet. Develop.* 3:102; GB 2200651; EP 0415731; EP 0345242; WO 89/02468; WO 89/05349; WO 89/09271; WO

90/02806; WO 90/07936; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; in U.S. 5,219,740; U.S. 4,405,712; U.S. 4,861,719; U.S. 4,980,289 and U.S. 4,777,127; in U.S. Serial No. 07/800,921; and in Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53:83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci USA* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

In other embodiments, gene transfer vectors can be constructed to encode a cytokine or other immunomodulatory molecule. For example, nucleic acid sequences encoding native IL-2 and gamma-interferon can be obtained as described in US Patent Nos. 4,738,927 and 5,326,859, respectively, while useful muteins of these proteins can be obtained as described in U.S. Patent No. 4,853,332. Nucleic acid sequences encoding the short and long forms of mCSF can be obtained as described in US Patent Nos. 4,847,201 and 4,879,227, respectively. In particular aspects of the invention, retroviral vectors expressing cytokine or immunomodulatory genes can be produced as described herein (for example, employing the packaging cell lines of the present invention) and in International Application No. PCT US 94/02951, entitled "Compositions and Methods for Cancer Immunotherapy."

Examples of suitable immunomodulatory molecules for use herein include the following: IL-1 and IL-2 (Karupiah et al. (1990) *J. Immunology* 144:290-298, Weber et al. (1987) *J. Exp. Med.* 166:1716-1733, Gansbacher et al. (1990) *J. Exp. Med.* 172:1217-1224, and U.S. Patent No. 4,738,927); IL-3 and IL-4 (Tepper et al. (1989) *Cell* 57:503-512, Golumbek et al. (1991) *Science* 254:713-716, and U.S. Patent No. 5,017,691); IL-5 and IL-6 (Brakenhof et al. (1987) *J. Immunol.* 139:4116-4121, and International Publication No. WO 90/06370); IL-7 (U.S. Patent No. 4,965,195); IL-8, IL-9, IL-10, IL-11, IL-12, and IL-13 (*Cytokine Bulletin*, Summer 1994); IL-14 and IL-15; alpha interferon (Finter et al. (1991) *Drugs* 42:749-765, U.S. Patent Nos. 4,892,743 and 4,966,843, International Publication No. WO 85/02862, Nagata et al. (1980) *Nature* 284:316-320, Familletti et al. (1981) *Methods in Enz.* 78:387-394, Twu et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:2046-2050, and Faktor et al. (1990) *Oncogene* 5:867-872); beta-interferon (Seif et al. (1991) *J. Virol.* 65:664-671); gamma-interferons (Radford et al. (1991) *The American Society of Hepatology*

20082015, Watanabe et al. (1989) *Proc. Natl. Acad. Sci. USA* **86**:9456-9460, Gansbacher et al. (1990) *Cancer Research* **50**:7820-7825, Maio et al. (1989) *Can. Immunol. Immunother.* **30**:34-42, and U.S. Patent Nos. 4,762,791 and 4,727,138); G-CSF (U.S. Patent Nos. 4,999,291 and 4,810,643); GM-CSF (International Publication No. WO 85/04188).

5 Immunomodulatory factors may also be agonists, antagonists, or ligands for these molecules. For example, soluble forms of receptors can often behave as antagonists for these types of factors, as can mutated forms of the factors themselves.

Nucleic acid molecules that encode the above-described substances, as well as  
10 other nucleic acid molecules that are advantageous for use within the present invention, may be readily obtained from a variety of sources, including, for example, depositories such as the American Type Culture Collection, or from commercial sources such as British Bio-Technology Limited (Cowley, Oxford England).

15 Representative examples include BBG 12 (containing the GM-CSF gene coding for the mature protein of 127 amino acids), BBG 6 (which contains sequences encoding gamma interferon), A.T.C.C. Deposit No. 39656 (which contains sequences encoding TNF), A.T.C.C. Deposit No. 20663 (which contains sequences encoding alpha-interferon), A.T.C.C. Deposit Nos. 31902, 31902 and 39517 (which contain sequences encoding beta-interferon), A.T.C.C. Deposit No. 67024 (which contains a  
20 sequence which encodes Interleukin-1b), A.T.C.C. Deposit Nos. 39405, 39452, 39516, 39626 and 39673 (which contain sequences encoding Interleukin-2), A.T.C.C. Deposit Nos. 59399, 59398, and 67326 (which contain sequences encoding Interleukin-3), A.T.C.C. Deposit No. 57592 (which contains sequences encoding Interleukin-4), A.T.C.C. Deposit Nos. 59394 and 59395 (which contain sequences  
25 encoding Interleukin-5), and A.T.C.C. Deposit No. 67153 (which contains sequences encoding Interleukin-6).

Plasmids containing cytokine genes or immunomodulatory genes (International Publication Nos. WO 94/02951 and WO) can be digested with appropriate restriction enzymes, and DNA fragments containing the particular gene of interest can be inserted into a gene transfer vector using standard molecular biology techniques. (See, e.g., Sambrook et al., *supra.*, or Ausbel et al. (eds) *Current Protocols in Molecular Biology*, Greene Publishing and Wiley-Interscience).

- Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. For example, plasmids which contain sequences that encode 5 altered cellular products may be obtained from a depository such as the A.T.C.C., or from commercial sources. Plasmids containing the nucleotide sequences of interest can be digested with appropriate restriction enzymes, and DNA fragments containing the nucleotide sequences can be inserted into a gene transfer vector using standard molecular biology techniques.
- 10 Alternatively, cDNA sequences for use with the present invention may be obtained from cells which express or contain the sequences, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA. Briefly, mRNA from a cell which expresses the gene of interest can be reverse 15 transcribed with reverse transcriptase using oligo-dT or random primers. The single stranded cDNA may then be amplified by PCR (see U.S. Patent Nos. 4,683,202, 4,683,195 and 4,800,159, see also *PCR Technology: Principles and Applications for DNA Amplification*, Erlich (ed.), Stockton Press, 1989)) using oligonucleotide primers complementary to sequences on either side of desired sequences.
- 20 The nucleotide sequence of interest can also be produced synthetically, rather than cloned, using a DNA synthesizer (e.g., an Applied Biosystems Model 392 DNA Synthesizer, available from ABI, Foster City, California). The nucleotide sequence can be designed with the appropriate codons for the expression product desired. The complete sequence is assembled from overlapping oligonucleotides prepared by 25 standard methods and assembled into a complete coding sequence. See, e.g., Edge (1981) *Nature* 292:756; Nambair et al. (1984) *Science* 223:1299; Jay et al. (1984) *J. Biol. Chem.* 259:6311.
- The synthetic expression cassettes of the present invention can be employed in the construction of packaging cell lines for use with retroviral vectors.
- 30 One type of retrovirus, the murine leukemia virus, or "MLV", has been widely utilized for gene therapy applications (see generally Mann et al. (*Cell* 33:153, 1993),

Cane and Mulligan (*Proc. Nat'l. Acad. Sci. USA* 81:6349, 1984), and Miller et al., *Human Gene Therapy* 1:5-14, 1990.

Lentiviral vectors typically, comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, a promoter operably linked to one or more genes of interest, an origin of second strand DNA synthesis and a 3' lentiviral LTR, wherein the lentiviral vector contains a nuclear transport element. The nuclear transport element may be located either upstream (5') or downstream (3') of a coding sequence of interest (for example, a synthetic Gag or Env expression cassette of the present invention). Within certain embodiments, the nuclear transport element is not RRE. Within one embodiment the packaging signal is an extended packaging signal. Within other embodiments the promoter is a tissue specific promoter, or, alternatively, a promoter such as CMV. Within other embodiments, the lentiviral vector further comprises an internal ribosome entry site.

A wide variety of lentiviruses may be utilized within the context of the present invention, including for example, lentiviruses selected from the group consisting of HIV, HIV-1, HIV-2, FIV and SIV.

In one embodiment of the present invention synthetic Gag-polymerase expression cassettes are provided comprising a promoter and a sequence encoding synthetic Gag-polymerase and at least one of vpr, vpu, nef or vif, wherein the promoter is operably linked to Gag-polymerase and vpr, vpu, nef or vif.

Within yet another aspect of the invention, host cells (eg., packaging cell lines) are provided which contain any of the expression cassettes described herein. For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence encoding synthetic Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Gag-polymerase. Packaging cell lines may further comprise a promoter and a sequence encoding tat, rev, or an envelope, wherein the promoter is operably linked to the sequence encoding tat, rev, Env or modified Env proteins. The packaging cell line may further comprise a sequence encoding any one or more of nef, vif, vpu or vpr.

In one embodiment, the expression cassette (carrying, for example, the synthetic Gag-polymerase) is stably integrated. The packaging cell line, upon

introduction of a lentiviral vector, typically produces particles. The promoter regulating expression of the synthetic expression cassette may be inducible. Typically, the packaging cell line, upon introduction of a lentiviral vector, produces particles that are essentially free of replication competent virus.

5       Packaging cell lines are provided comprising an expression cassette which directs the expression of a synthetic *Gag-polymerase* gene or comprising an expression cassette which directs the expression of a synthetic Env genes described herein. (See, also, Andre, S., et al., *Journal of Virology* 72(2):1497-1503, 1998; Haas, J., et al., *Current Biology* 6(3):315-324, 1996) for a description of other modified Env  
10 sequences). A lentiviral vector is introduced into the packaging cell line to produce a vector producing cell line.

As noted above, lentiviral vectors can be designed to carry or express a selected gene(s) or sequences of interest. Lentiviral vectors may be readily constructed from a wide variety of lentiviruses (*see* RNA Tumor Viruses, Second  
15 Edition, Cold Spring Harbor Laboratory, 1985). Representative examples of lentiviruses included HIV, HIV-1, HIV-2, FIV and SIV. Such lentiviruses may either be obtained from patient isolates, or, more preferably, from depositories or collections such as the American Type Culture Collection, or isolated from known sources using available techniques.

20       Portions of the lentiviral gene delivery vectors (or vehicles) may be derived from different viruses. For example, in a given recombinant lentiviral vector, LTRs may be derived from an HIV, a packaging signal from SIV, and an origin of second strand synthesis from HrV-2. Lentiviral vector constructs may comprise a 5' lentiviral LTR, a tRNA binding site, a packaging signal, one or more heterologous sequences,  
25 an origin of second strand DNA synthesis and a 3' LTR, wherein said lentiviral vector contains a nuclear transport element that is not RRE.

Briefly, Long Terminal Repeats ("LTRs") are subdivided into three elements, designated U5, R and U3. These elements contain a variety of signals which are responsible for the biological activity of a retrovirus, including for example, promoter  
30 and enhancer elements which are located within U3. LTRs may be readily identified in the provirus (integrated DNA form) due to their precise duplication at either end of the genome. As utilized herein, a 5' LTR should be understood to include a 5'

promoter element and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector. The 3' LTR should be understood to include a polyadenylation signal, and sufficient LTR sequence to allow reverse transcription and integration of the DNA form of the vector.

5       The tRNA binding site and origin of second strand DNA synthesis are also important for a retrovirus to be biologically active, and may be readily identified by one of skill in the art. For example, retroviral tRNA binds to a tRNA binding site by Watson-Crick base pairing, and is carried with the retrovirus genome into a viral particle. The tRNA is then utilized as a primer for DNA synthesis by reverse  
10 transcriptase. The tRNA binding site may be readily identified based upon its location just downstream from the 5'LTR. Similarly, the origin of second strand DNA synthesis is, as its name implies, important for the second strand DNA synthesis of a retrovirus. This region, which is also referred to as the poly-purine tract, is located just upstream of the 3'LTR.

15       In addition to a 5' and 3' LTR, tRNA binding site, and origin of second strand DNA synthesis, recombinant retroviral vector constructs may also comprise a packaging signal, as well as one or more genes or coding sequences of interest. In addition, the lentiviral vectors have a nuclear transport element which, in preferred embodiments is not RRE. Representative examples of suitable nuclear transport  
20 elements include the element in Rous sarcoma virus (Ogert, et al., *J Virol* 70, 3834-3843, 1996), the element in Rous sarcoma virus (Liu & Mertz, *Genes & Dev.*, 9, 1766-1789, 1995) and the element in the genome of simian retrovirus type I (Zolotukhin, et al., *J Virol.* 68, 7944-7952, 1994). Other potential elements include the elements in the histone gene (Kedes, *Annu. Rev. Biochem.* 48, 837-870, 1970), the  
25  $\alpha$ -interferon gene (Nagata et al., *Nature* 287, 401-408, 1980), the  $\beta$ -adrenergic receptor gene (Koilkka, et al., *Nature* 329, 75-79, 1987), and the c-Jun gene (Hattorie, et al., *Proc. Natl. Acad. Sci. USA* 85, 9148-9152, 1988).

Recombinant lentiviral vector constructs typically lack both *Gag-polymerase* and *Env* coding sequences. Recombinant lentiviral vector typically contain less than  
30 20, preferably 15, more preferably 10, and most preferably 8 consecutive nucleotides found in *Gag-polymerase* and *Env* genes. One advantage of the present invention is that the synthetic *Gag-polymerase* expression cassettes, which can be used to

construct packaging cell lines for the recombinant retroviral vector constructs, have little homology to wild-type Gag-polymerase sequences and thus considerably reduce or eliminate the possibility of homologous recombination between the synthetic and wild-type sequences.

- 5       Lentiviral vectors may also include tissue-specific promoters to drive expression of one or more genes or sequences of interest.

Lentiviral vector constructs may be generated such that more than one gene of interest is expressed. This may be accomplished through the use of di- or oligo-cistronic cassettes (e.g., where the coding regions are separated by 80 nucleotides or 10 less, *see generally* Levin et al., *Gene* 108:167-174, 1991), or through the use of Internal Ribosome Entry Sites ("IRES").

Packaging cell lines suitable for use with the above described recombinant retroviral vector constructs may be readily prepared given the disclosure provided herein. Briefly, the parent cell line from which the packaging cell line is derived can 15 be selected from a variety of mammalian cell lines, including for example, 293, RD, COS-7, CHO, BHK, VERO, HT1080, and myeloma cells.

After selection of a suitable host cell for the generation of a packaging cell line, one or more expression cassettes are introduced into the cell line in order to complement or supply in *trans* components of the vector which have been deleted.

20       Representative examples of suitable expression cassettes have been described herein and include synthetic Env, synthetic Gag, synthetic Gag-protease, and synthetic Gag-polymerase expression cassettes, which comprise a promoter and a sequence encoding, e.g., Gag-polymerase and at least one of vpr, vpu, nef or vif, wherein the promoter is operably linked to Gag-polymerase and vpr, vpu, nef or vif. As described 25 above, the native and/or modified Env coding sequences may also be utilized in these expression cassettes.

Utilizing the above-described expression cassettes, a wide variety of packaging cell lines can be generated. For example, within one aspect packaging cell line are provided comprising an expression cassette that comprises a sequence 30 encoding synthetic Gag-polymerase, and a nuclear transport element, wherein the promoter is operably linked to the sequence encoding Gag-polymerase. Within other aspects, packaging cell lines are provided comprising a promoter and a sequence

encoding tat, rev, Env, or other HIV antigens or epitopes derived therefrom, wherein the promoter is operably linked to the sequence encoding tat, rev, Env, or the HIV antigen or epitope. Within further embodiments, the packaging cell line may comprise a sequence encoding any one or more of nef, vif, vpu or vpr. For example, 5 the packaging cell line may contain only nef, vif, vpu, or vpr alone, nef and vif, nef and vpu, nef and vpr, vif and vpu, vif and vpr, vpu and vpr, nef vif and vpu, nef vif and vpr, nef vpu and vpr, vvir vpu and vpr, or, all four of nef vif vpu and vpr.

In one embodiment, the expression cassette is stably integrated. Within another embodiment, the packaging cell line, upon introduction of a lentiviral vector, 10 produces particles. Within further embodiments the promoter is inducible. Within certain preferred embodiments of the invention, the packaging cell line, upon introduction of a lentiviral vector, produces particles that are free of replication competent virus.

The synthetic cassettes containing optimized coding sequences are transfected 15 into a selected cell line. Transfected cells are selected that (i) carry, typically, integrated, stable copies of the Gag, Pol, and Env coding sequences, and (ii) are expressing acceptable levels of these polypeptides (expression can be evaluated by methods known in the prior art, e.g., see Examples 1-4). The ability of the cell line to produce VLPs may also be verified.

20 A sequence of interest is constructed into a suitable viral vector as discussed above. This defective virus is then transfected into the packaging cell line. The packaging cell line provides the viral functions necessary for producing virus-like particles into which the defective viral genome, containing the sequence of interest, are packaged. These VLPs are then isolated and can be used, for example, in gene 25 delivery or gene therapy.

Further, such packaging cell lines can also be used to produce VLPs alone, 30 which can, for example, be used as adjuvants for administration with other antigens or in vaccine compositions. Also, co-expression of a selected sequence of interest encoding a polypeptide (for example, an antigen) in the packaging cell line can also result in the entrapment and/or association of the selected polypeptide in/with the VLPs.

### 2.3 DNA IMMUNIZATION AND GENE DELIVERY

A variety of HIV polypeptide antigens, particularly Type C HIV antigens, can be used in the practice of the present invention. HIV antigens can be included in DNA immunization constructs containing, for example, a synthetic Gag expression cassette fused in-frame to a coding sequence for the polypeptide antigen, where expression of the construct results in VLPs presenting the antigen of interest.

HIV antigens of particular interest to be used in the practice of the present invention include tat, rev, nef, vif, vpu, vpr, and other HIV antigens or epitopes derived therefrom. For example, the packaging cell line may contain only nef, and 10 HIV-1 (also known as HTLV-III, LAV, ARV, etc.), including, but not limited to, antigens such as gp120, gp41, gp160 (both native and modified); Gag; and pol from a variety of isolates including, but not limited to, HIV<sub>IIIb</sub>, HIV<sub>SF2</sub>, HIV-1<sub>SF162</sub>, HIV-1<sub>SF170</sub>, HIV<sub>LAV</sub>, HIV<sub>LAI</sub>, HIV<sub>MN</sub>, HIV-1<sub>CM235</sub>, HIV-1<sub>US4</sub>, other HIV-1 strains from diverse subtypes(e.g., subtypes, A through G, and O), HIV-2 strains and diverse subtypes 15 (e.g., HIV-2<sub>UC1</sub> and HIV-2<sub>UC2</sub>). See, e.g., Myers, et al., Los Alamos Database, Los Alamos National Laboratory, Los Alamos, New Mexico; Myers, et al., *Human Retroviruses and Aids, 1990*, Los Alamos, New Mexico: Los Alamos National Laboratory.

To evaluate efficacy, DNA immunization using synthetic expression cassettes 20 of the present invention can be performed, for instance as described in Example 4. Mice are immunized with both the Gag (and/or Env) synthetic expression cassette and the Gag (and/or Env) wild type expression cassette. Mouse immunizations with plasmid-DNAs will show that the synthetic expression cassettes provide a clear improvement of immunogenicity relative to the native expression cassettes. Also, the 25 second boost immunization will induce a secondary immune response, for example, after approximately two weeks. Further, the results of CTL assays will show increased potency of synthetic Gag (and/or Env) expression cassettes for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

It is readily apparent that the subject invention can be used to mount an 30 immune response to a wide variety of antigens and hence to treat or prevent a HIV infection, particularly Type C HIV infection.

### 2.3.1 DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF THE PRESENT INVENTION

Polynucleotide sequences coding for the above-described molecules can be obtained using recombinant methods, such as by screening cDNA and genomic libraries from cells expressing the gene, or by deriving the gene from a vector known to include the same. Furthermore, the desired gene can be isolated directly from cells and tissues containing the same, using standard techniques, such as phenol extraction and PCR of cDNA or genomic DNA. See, e.g., Sambrook et al., *supra*, for a description of techniques used to obtain and isolate DNA. The gene of interest can also be produced synthetically, rather than cloned. The nucleotide sequence can be designed with the appropriate codons for the particular amino acid sequence desired. In general, one will select preferred codons for the intended host in which the sequence will be expressed. The complete sequence is assembled from overlapping oligonucleotides prepared by standard methods and assembled into a complete coding sequence. See, e.g., Edge, *Nature* (1981) 292:756; Nambair et al., *Science* (1984) 223:1299; Jay et al., *J. Biol. Chem.* (1984) 259:6311; Stemmer, W.P.C., (1995) *Gene* 164:49-53.

Next, the gene sequence encoding the desired antigen can be inserted into a vector containing a synthetic Gag or synthetic Env expression cassette of the present invention. The antigen is inserted into the synthetic Gag coding sequence such that when the combined sequence is expressed it results in the production of VLPs comprising the Gag polypeptide and the antigen of interest, e.g., Env (native or modified) or other antigen derived from HIV. Insertions can be made within the coding sequence or at either end of the coding sequence (5', amino terminus of the expressed Gag polypeptide; or 3', carboxy terminus of the expressed Gag polypeptide)(Wagner, R., et al., *Arch Virol.* 127:117-137, 1992; Wagner, R., et al., *Virology* 200:162-175, 1994; Wu, X., et al., *J. Virol.* 69(6):3389-3398, 1995; Wang, C-T., et al., *Virology* 200:524-534, 1994; Chazal, N., et al., *Virology* 68(1):111-122, 1994; Griffiths, J.C., et al., *J. Virol.* 67(6):3191-3198, 1993; Reicin, A.S., et al., *J. Virol.* 69(2):642-650, 1995).

Up to 50% of the coding sequences of p55Gag can be deleted without affecting the assembly to virus-like particles and expression efficiency (Borsetti, A.,

et al., *J. Virol.* 72(11):9313-9317, 1998; Gamier, L., et al., *J Virol* 72(6):4667-4677, 1998; Zhang, Y., et al., *J Virol* 72(3):1782-1789, 1998; Wang, C., et al., *J Virol* 72(10): 7950-7959, 1998). In one embodiment of the present invention, immunogenicity of the high level expressing synthetic Gag expression cassettes can be increased by the insertion of different structural or non-structural HIV antigens, multiepitope cassettes, or cytokine sequences into deleted regions of Gag sequence. Such deletions may be generated following the teachings of the present invention and information available to one of ordinary skill in the art. One possible advantage of this approach, relative to using full-length sequences fused to heterologous polypeptides, can be higher expression/secretion efficiency of the expression product.

When sequences are added to the amino terminal end of Gag, the polynucleotide can contain coding sequences at the 5' end that encode a signal for addition of a myristic moiety to the Gag-containing polypeptide (e.g., sequences that encode Met-Gly).

The ability of Gag-containing polypeptide constructs to form VLPs can be empirically determined following the teachings of the present specification.

Gag/antigen (e.g., Gag/Env) synthetic expression cassettes include control elements operably linked to the coding sequence, which allow for the expression of the gene *in vivo* in the subject species. For example, typical promoters for mammalian cell expression include the SV40 early promoter, a CMV promoter such as the CMV immediate early promoter, the mouse mammary tumor virus LTR promoter, the adenovirus major late promoter (Ad MLP), and the herpes simplex virus promoter, among others. Other nonviral promoters, such as a promoter derived from the murine metallothionein gene, will also find use for mammalian expression.

Typically, transcription termination and polyadenylation sequences will also be present, located 3' to the translation stop codon. Preferably, a sequence for optimization of initiation of translation, located 5' to the coding sequence, is also present. Examples of transcription terminator/polyadenylation signals include those derived from SV40, as described in Sambrook et al., *supra*, as well as a bovine growth hormone terminator sequence.

Enhancer elements may also be used herein to increase expression levels of the mammalian constructs. Examples include the SV40 early gene enhancer, as

described in Dijkema et al., *EMBO J.* (1985) 4:761, the enhancer/promoter derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus, as described in Gorman et al., *Proc. Natl. Acad. Sci. USA* (1982b) 79:6777 and elements derived from human CMV, as described in Boshart et al., *Cell* (1985) 41:521, such as 5 elements included in the CMV intron A sequence.

Furthermore, plasmids can be constructed which include a chimeric antigen-coding gene sequences, encoding, e.g., multiple antigens/epitopes of interest, for example derived from more than one viral isolate.

Typically the antigen coding sequences precede or follow the synthetic coding 10 sequence and the chimeric transcription unit will have a single open reading frame encoding both the antigen of interest and the synthetic Gag coding sequences.

Alternatively, multi-cistronic cassettes (e.g., bi-cistronic cassettes) can be constructed allowing expression of multiple antigens from a single mRNA using the EMCV IRES, or the like.

Once complete, the constructs are used for nucleic acid immunization using 15 standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Patent Nos. 5,399,346, 5,580,859, 5,589,466. Genes can be delivered either directly to the vertebrate subject or, alternatively, delivered *ex vivo*, to cells derived from the subject and the cells reimplanted in the subject.

A number of viral based systems have been developed for gene transfer into 20 mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. Selected sequences can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of 25 retroviral systems have been described (U.S. Patent No. 5,219,740; Miller and Rosman, *BioTechniques* (1989) 7:980-990; Miller, A.D., *Human Gene Therapy* (1990) 1:5-14; Scarpa et al., *Virology* (1991) 180:849-852; Burns et al., *Proc. Natl. Acad. Sci. USA* (1993) 90:8033-8037; and Boris-Lawrie and Temin, *Cur. Opin. Genet. Develop.* (1993) 3:102-109.

30 A number of adenovirus vectors have also been described. Unlike retroviruses which integrate into the host genome, adenoviruses persist extrachromosomally thus minimizing the risks associated with insertional mutagenesis (Haj-Ahmad and

Graham, *J. Virol.* (1986) 57:267-274; Bett et al., *J. Virol.* (1993) 67:5911-5921; Mittereder et al., *Human Gene Therapy* (1994) 5:717-729; Seth et al., *J. Virol.* (1994) 68:933-940; Barr et al., *Gene Therapy* (1994) 1:51-58; Berkner, K.L. *BioTechniques* (1988) 6:616-629; and Rich et al., *Human Gene Therapy* (1993) 4:461-476).

5        Additionally, various adeno-associated virus (AAV) vector systems have been developed for gene delivery. AAV vectors can be readily constructed using techniques well known in the art. See, e.g., U.S. Patent Nos. 5,173,414 and 5,139,941; International Publication Nos. WO 92/01070 (published 23 January 1992) and WO 93/03769 (published 4 March 1993); Lebkowski et al., *Molec. Cell. Biol.* (1988) 8:3988-3996; Vincent et al., *Vaccines* 90 (1990) (Cold Spring Harbor Laboratory Press); Carter, B.J. *Current Opinion in Biotechnology* (1992) 3:533-539; Muzyczka, N. *Current Topics in Microbiol. and Immunol.* (1992) 158:97-129; Kotin, R.M. *Human Gene Therapy* (1994) 5:793-801; Shelling and Smith, *Gene Therapy* (1994) 1:165-169; and Zhou et al., *J. Exp. Med.* (1994) 179:1867-1875.

10      Another vector system useful for delivering the polynucleotides of the present invention is the enterically administered recombinant poxvirus vaccines described by Small, Jr., P.A., et al. (U.S. Patent No. 5,676,950, issued October 14, 1997).

15      Additional viral vectors which will find use for delivering the nucleic acid molecules encoding the antigens of interest include those derived from the pox family 20 of viruses, including vaccinia virus and avian poxvirus. By way of example, vaccinia virus recombinants expressing the genes can be constructed as follows. The DNA encoding the particular synthetic Gag/ or Env/antigen coding sequence is first inserted into an appropriate vector so that it is adjacent to a vaccinia promoter and flanking vaccinia DNA sequences, such as the sequence encoding thymidine kinase (TK).

25      This vector is then used to transfet cells which are simultaneously infected with vaccinia. Homologous recombination serves to insert the vaccinia promoter plus the gene encoding the coding sequences of interest into the viral genome. The resulting TK recombinant can be selected by culturing the cells in the presence of 5-bromodeoxyuridine and picking viral plaques resistant thereto.

30      Alternatively, avipoxviruses, such as the fowlpox and canarypox viruses, can also be used to deliver the genes. Recombinant avipox viruses, expressing immunogens from mammalian pathogens, are known to confer protective immunity

when administered to non-avian species. The use of an avipox vector is particularly desirable in human and other mammalian species since members of the avipox genus can only productively replicate in susceptible avian species and therefore are not infective in mammalian cells. Methods for producing recombinant avipoxviruses are known in the art and employ genetic recombination, as described above with respect to the production of vaccinia viruses. See, e.g., WO 91/12882; WO 89/03429; and WO 92/03545.

5 Molecular conjugate vectors, such as the adenovirus chimeric vectors described in Michael et al., *J. Biol. Chem.* (1993) 268:6866-6869 and Wagner et al.,  
10 *Proc. Natl. Acad. Sci. USA* (1992) 89:6099-6103, can also be used for gene delivery.

Members of the Alphavirus genus, such as, but not limited to, vectors derived from the Sindbis, Semliki Forest, and Venezuelan Equine Encephalitis viruses, will also find use as viral vectors for delivering the polynucleotides of the present invention (for example, a synthetic Gag-polypeptide encoding expression cassette).  
15 For a description of Sindbis-virus derived vectors useful for the practice of the instant methods, see, Dubensky et al., *J. Virol.* (1996) 70:508-519; and International Publication Nos. WO 95/07995 and WO 96/17072; as well as, Dubensky, Jr., T.W., et al., U.S. Patent No. 5,843,723, issued December 1, 1998, and Dubensky, Jr., T.W., U.S. Patent No. 5,789,245, issued August 4, 1998.

20 A vaccinia based infection/transfection system can be conveniently used to provide for inducible, transient expression of the coding sequences of interest in a host cell. In this system, cells are first infected *in vitro* with a vaccinia virus recombinant that encodes the bacteriophage T7 RNA polymerase. This polymerase displays exquisite specificity in that it only transcribes templates bearing T7  
25 promoters. Following infection, cells are transfected with the polynucleotide of interest, driven by a T7 promoter. The polymerase expressed in the cytoplasm from the vaccinia virus recombinant transcribes the transfected DNA into RNA which is then translated into protein by the host translational machinery. The method provides for high level, transient, cytoplasmic production of large quantities of RNA and its  
30 translation products. See, e.g., Elroy-Stein and Moss, *Proc. Natl. Acad. Sci. USA* (1990) 87:6743-6747; Fuerst et al., *Proc. Natl. Acad. Sci. USA* (1986) 83:8122-8126.

As an alternative approach to infection with vaccinia or avipox virus recombinants, or to the delivery of genes using other viral vectors, an amplification system can be used that will lead to high level expression following introduction into host cells. Specifically, a T7 RNA polymerase promoter preceding the coding region 5 for T7 RNA polymerase can be engineered. Translation of RNA derived from this template will generate T7 RNA polymerase which in turn will transcribe more template. Concomitantly, there will be a cDNA whose expression is under the control of the T7 promoter. Thus, some of the T7 RNA polymerase generated from translation of the amplification template RNA will lead to transcription of the desired 10 gene. Because some T7 RNA polymerase is required to initiate the amplification, T7 RNA polymerase can be introduced into cells along with the template(s) to prime the transcription reaction. The polymerase can be introduced as a protein or on a plasmid encoding the RNA polymerase. For a further discussion of T7 systems and their use for transforming cells, see, e.g., International Publication No. WO 94/26911; Studier 15 and Moffatt, *J. Mol. Biol.* (1986) 189:113-130; Deng and Wolff, *Gene* (1994) 143:245-249; Gao et al., *Biochem. Biophys. Res. Commun.* (1994) 200:1201-1206; Gao and Huang, *Nuc. Acids Res.* (1993) 21:2867-2872; Chen et al., *Nuc. Acids Res.* (1994) 22:2114-2120; and U.S. Patent No. 5,135,855.

A synthetic Gag- and/or Env-containing expression cassette of interest can 20 also be delivered without a viral vector. For example, the synthetic expression cassette can be packaged in liposomes prior to delivery to the subject or to cells derived therefrom. Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed DNA to lipid preparation can vary but will generally be around 1:1 (mg 25 DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight, *Biochim. Biophys. Acta* (1991) 1097:1-17; Straubinger et al., in *Methods of Enzymology* (1983), Vol. 101, pp. 512-527.

Liposomal preparations for use in the present invention include cationic 30 (positively charged), anionic (negatively charged) and neutral preparations, with cationic liposomes particularly preferred. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., *Proc. Natl. Acad. Sci.*

USA (1987) 84:7413-7416); mRNA (Malone et al., *Proc. Natl. Acad. Sci. USA* (1989) 86:6077-6081); and purified transcription factors (Debs et al., *J. Biol. Chem.* (1990) 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy]propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner et al., *Proc. Natl. Acad. Sci. USA* (1987) 84:7413-7416). Other commercially available lipids include (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g., Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; PCT Publication No. WO 90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as, from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See, e.g., Straubinger et al., in METHODS OF IMMUNOLOGY (1983), Vol. 101, pp. 512-527; Szoka et al., *Proc. Natl. Acad. Sci. USA* (1978) 75:4194-4198; Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* (1979) 17:77; Deamer and Bangham, *Biochim. Biophys. Acta* (1976) 443:629; Ostro et al., *Biochem. Biophys. Res. Commun.* (1977) 76:836; Fraley et al., *Proc. Natl. Acad. Sci. USA* (1979) 76:3348; Enoch and Strittmatter, *Proc. Natl. Acad. Sci. USA* (1979) 76:145; Fraley et al., *J. Biol. Chem.* (1980) 255:10431; Szoka and Papahadjopoulos, *Proc. Natl. Acad. Sci. USA* (1978) 75:145; and Schaefer-Ridder et al., *Science* (1982) 215:166.

The DNA and/or protein antigen(s) can also be delivered in cochleate lipid compositions similar to those described by Papahadjopoulos et al., *Biochem. Biophys. Acta.* (1975) 394:483-491. See, also, U.S. Patent Nos. 4,663,161 and 4,871,488.

The synthetic expression cassette of interest may also be encapsulated, 5 adsorbed to, or associated with, particulate carriers. Such carriers present multiple copies of a selected antigen to the immune system and promote trapping and retention of antigens in local lymph nodes. The particles can be phagocytosed by macrophages and can enhance antigen presentation through cytokine release. Examples of particulate carriers include those derived from polymethyl methacrylate polymers, as 10 well as microparticles derived from poly(lactides) and poly(lactide-co-glycolides), known as PLG. See, e.g., Jeffery et al., *Pharm. Res.* (1993) 10:362-368; McGee JP, et al., *J Microencapsul.* 14(2):197-210, 1997; O'Hagan DT, et al., *Vaccine* 11(2):149-54, 1993. Suitable microparticles may also be manufactured in the presence of charged detergents, such as anionic or cationic detergents, to yield microparticles with a 15 surface having a net negative or a net positive charge. For example, microparticles manufactured with anionic detergents, such as hexadecyltrimethylammonium bromide (CTAB), i.e. CTAB-PLG microparticles, adsorb negatively charged macromolecules, such as DNA. (see, e.g., Int'l Application Number PCT/US99/17308).

Furthermore, other particulate systems and polymers can be used for the *in* 20 *vivo* or *ex vivo* delivery of the gene of interest. For example, polymers such as polylysine, polyarginine, polyornithine, spermine, spermidine, as well as conjugates of these molecules, are useful for transferring a nucleic acid of interest. Similarly, DEAE dextran-mediated transfection, calcium phosphate precipitation or precipitation using other insoluble inorganic salts, such as strontium phosphate, aluminum silicates 25 including bentonite and kaolin, chromic oxide, magnesium silicate, talc, and the like, will find use with the present methods. See, e.g., Felgner, P.L., *Advanced Drug Delivery Reviews* (1990) 5:163-187, for a review of delivery systems useful for gene transfer. Peptoids (Zuckerman, R.N., et al., U.S. Patent No. 5,831,005, issued November 3, 1998) may also be used for delivery of a construct of the present 30 invention.

Additionally, biolistic delivery systems employing particulate carriers such as gold and tungsten, are especially useful for delivering synthetic expression cassettes

- of the present invention. The particles are coated with the synthetic expression cassette(s) to be delivered and accelerated to high velocity, generally under a reduced atmosphere, using a gun powder discharge from a "gene gun." For a description of such techniques, and apparatuses useful therefore, see, e.g., U.S. Patent Nos. 5 4,945,050; 5,036,006; 5,100,792; 5,179,022; 5,371,015; and 5,478,744. Also, needle-less injection systems can be used (Davis, H.L., et al, *Vaccine* 12:1503-1509, 1994; Bioject, Inc., Portland, OR).

Recombinant vectors carrying a synthetic expression cassette of the present invention are formulated into compositions for delivery to the vertebrate subject. 10 These compositions may either be prophylactic (to prevent infection) or therapeutic (to treat disease after infection). The compositions will comprise a "therapeutically effective amount" of the gene of interest such that an amount of the antigen can be produced *in vivo* so that an immune response is generated in the individual to which it is administered. The exact amount necessary will vary depending on the subject 15 being treated; the age and general condition of the subject to be treated; the capacity of the subject's immune system to synthesize antibodies; the degree of protection desired; the severity of the condition being treated; the particular antigen selected and its mode of administration, among other factors. An appropriate effective amount can be readily determined by one of skill in the art. Thus, a "therapeutically effective 20 amount" will fall in a relatively broad range that can be determined through routine trials.

The compositions will generally include one or more "pharmaceutically acceptable excipients or vehicles" such as water, saline, glycerol, polyethyleneglycol, hyaluronic acid, ethanol, etc. Additionally, auxiliary substances, such as wetting or 25 emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Certain facilitators of nucleic acid uptake and/or expression can also be included in the compositions or coadministered, such as, but not limited to, bupivacaine, cardiotoxin and sucrose.

Once formulated, the compositions of the invention can be administered 30 directly to the subject (e.g., as described above) or, alternatively, delivered *ex vivo*, to cells derived from the subject, using methods such as those described above. For example, methods for the *ex vivo* delivery and reimplantation of transformed cells into

a subject are known in the art and can include, e.g., dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, lipofectamine and LT-1 mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) (with or without the corresponding antigen) in liposomes, and direct 5 microinjection of the DNA into nuclei.

Direct delivery of synthetic expression cassette compositions *in vivo* will generally be accomplished with or without viral vectors, as described above, by injection using either a conventional syringe or a gene gun, such as the Accell® gene delivery system (PowderJect Technologies, Inc., Oxford, England). The constructs 10 can be injected either subcutaneously, epidermally, intradermally, intramucosally such as nasally, rectally and vaginally, intraperitoneally, intravenously, orally or intramuscularly. Delivery of DNA into cells of the epidermis is particularly preferred as this mode of administration provides access to skin-associated lymphoid cells and provides for a transient presence of DNA in the recipient. Other modes of 15 administration include oral and pulmonary administration, suppositories, needle-less injection, transcutaneous and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. Administration of nucleic acids may also be combined with administration of peptides or other substances.

20           **2.3.2 EX VIVO DELIVERY OF THE SYNTHETIC EXPRESSION CASSETTES OF  
THE PRESENT INVENTION**

In one embodiment, T cells, and related cell types (including but not limited to 25 antigen presenting cells, such as, macrophage, monocytes, lymphoid cells, dendritic cells, B-cells, T-cells, stem cells, and progenitor cells thereof), can be used for *ex vivo* delivery of the synthetic expression cassettes of the present invention. T cells can be isolated from peripheral blood lymphocytes (PBLs) by a variety of procedures known to those skilled in the art. For example, T cell populations can be "enriched" from a population of PBLs through the removal of accessory and B cells. In particular, T cell enrichment can be accomplished by the elimination of non-T cells using anti-MHC 30 class II monoclonal antibodies. Similarly, other antibodies can be used to deplete specific populations of non-T cells. For example, anti-Ig antibody molecules can be

used to deplete B cells and anti-MacI antibody molecules can be used to deplete macrophages.

T cells can be further fractionated into a number of different subpopulations by techniques known to those skilled in the art. Two major subpopulations can be 5 isolated based on their differential expression of the cell surface markers CD4 and CD8. For example, following the enrichment of T cells as described above, CD4<sup>+</sup> cells can be enriched using antibodies specific for CD4 (see Coligan et al., *supra*). The antibodies may be coupled to a solid support such as magnetic beads. Conversely, CD8<sup>+</sup> cells can be enriched through the use of antibodies specific for 10 CD4 (to remove CD4<sup>+</sup> cells), or can be isolated by the use of CD8 antibodies coupled to a solid support. CD4 lymphocytes from HIV-1 infected patients can be expanded *ex vivo*, before or after transduction as described by Wilson et. al. (1995) *J. Infect. Dis.* 172:88.

Following purification of T cells, a variety of methods of genetic modification 15 known to those skilled in the art can be performed using non-viral or viral-based gene transfer vectors constructed as described herein. For example, one such approach involves transduction of the purified T cell population with vector-containing supernatant of cultures derived from vector producing cells. A second approach involves co-cultivation of an irradiated monolayer of vector-producing cells with the 20 purified T cells. A third approach involves a similar co-cultivation approach; however, the purified T cells are pre-stimulated with various cytokines and cultured 48 hours prior to the co-cultivation with the irradiated vector producing cells. Pre-stimulation prior to such transduction increases effective gene transfer (Nolta et al. (1992) *Exp. Hematol.* 20:1065). Stimulation of these cultures to proliferate also 25 provides increased cell populations for re-infusion into the patient. Subsequent to co-cultivation, T cells are collected from the vector producing cell monolayer, expanded, and frozen in liquid nitrogen.

Gene transfer vectors, containing one or more synthetic expression cassette of 30 the present invention (associated with appropriate control elements for delivery to the isolated T cells) can be assembled using known methods.

Selectable markers can also be used in the construction of gene transfer vectors. For example, a marker can be used which imparts to a mammalian cell

transduced with the gene transfer vector resistance to a cytotoxic agent. The cytotoxic agent can be, but is not limited to, neomycin, aminoglycoside, tetracycline, chloramphenicol, sulfonamide, actinomycin, netropsin, distamycin A, anthracycline, or pyrazinamide. For example, neomycin phosphotransferase II imparts resistance to the neomycin analogue geneticin (G418).

The T cells can also be maintained in a medium containing at least one type of growth factor prior to being selected. A variety of growth factors are known in the art which sustain the growth of a particular cell type. Examples of such growth factors are cytokine mitogens such as rIL-2, IL-10, IL-12, and IL-15, which promote growth and activation of lymphocytes. Certain types of cells are stimulated by other growth factors such as hormones, including human chorionic gonadotropin (hCG) and human growth hormone. The selection of an appropriate growth factor for a particular cell population is readily accomplished by one of skill in the art.

For example, white blood cells such as differentiated progenitor and stem cells are stimulated by a variety of growth factors. More particularly, IL-3, IL-4, IL-5, IL-6, IL-9, GM-CSF, M-CSF, and G-CSF, produced by activated  $T_H$  and activated macrophages, stimulate myeloid stem cells, which then differentiate into pluripotent stem cells, granulocyte-monocyte progenitors, eosinophil progenitors, basophil progenitors, megakaryocytes, and erythroid progenitors. Differentiation is modulated by growth factors such as GM-CSF, IL-3, IL-6, IL-11, and EPO.

Pluripotent stem cells then differentiate into lymphoid stem cells, bone marrow stromal cells, T cell progenitors, B cell progenitors, thymocytes,  $T_H$  Cells,  $T_C$  cells, and B cells. This differentiation is modulated by growth factors such as IL-3, IL-4, IL-6, IL-7, GM-CSF, M-CSF, G-CSF, IL-2, and IL-5.

Granulocyte-monocyte progenitors differentiate to monocytes, macrophages, and neutrophils. Such differentiation is modulated by the growth factors GM-CSF, M-CSF, and IL-8. Eosinophil progenitors differentiate into eosinophils. This process is modulated by GM-CSF and IL-5.

The differentiation of basophil progenitors into mast cells and basophils is modulated by GM-CSF, IL-4, and IL-9. Megakaryocytes produce platelets in response to GM-CSF, EPO, and IL-6. Erythroid progenitor cells differentiate into red blood cells in response to EPO.

Thus, during activation by the CD3-binding agent, T cells can also be contacted with a mitogen, for example a cytokine such as IL-2. In particularly preferred embodiments, the IL-2 is added to the population of T cells at a concentration of about 50 to 100 µg/ml. Activation with the CD3-binding agent can 5 be carried out for 2 to 4 days.

Once suitably activated, the T cells are genetically modified by contacting the same with a suitable gene transfer vector under conditions that allow for transfection of the vectors into the T cells. Genetic modification is carried out when the cell density of the T cell population is between about  $0.1 \times 10^6$  and  $5 \times 10^6$ , preferably 10 between about  $0.5 \times 10^6$  and  $2 \times 10^6$ . A number of suitable viral and nonviral-based gene transfer vectors have been described for use herein.

After transduction, transduced cells are selected away from non-transduced cells using known techniques. For example, if the gene transfer vector used in the transduction includes a selectable marker which confers resistance to a cytotoxic 15 agent, the cells can be contacted with the appropriate cytotoxic agent, whereby non-transduced cells can be negatively selected away from the transduced cells. If the selectable marker is a cell surface marker, the cells can be contacted with a binding agent specific for the particular cell surface marker, whereby the transduced cells can be positively selected away from the population. The selection step can also entail 20 fluorescence-activated cell sorting (FACS) techniques, such as where FACS is used to select cells from the population containing a particular surface marker, or the selection step can entail the use of magnetically responsive particles as retrievable supports for target cell capture and/or background removal.

More particularly, positive selection of the transduced cells can be performed 25 using a FACS cell sorter (e.g. a FACSVantage™ Cell Sorter, Becton Dickinson Immunocytometry Systems, San Jose, CA) to sort and collect transduced cells expressing a selectable cell surface marker. Following transduction, the cells are stained with fluorescent-labeled antibody molecules directed against the particular cell surface marker. The amount of bound antibody on each cell can be measured by 30 passing droplets containing the cells through the cell sorter. By imparting an electromagnetic charge to droplets containing the stained cells, the transduced cells can be separated from other cells. The positively selected cells are then harvested in

sterile collection vessels. These cell sorting procedures are described in detail, for example, in the FACS Vantage™ Training Manual, with particular reference to sections 3-11 to 3-28 and 10-1 to 10-17.

- Positive selection of the transduced cells can also be performed using
- 5 magnetic separation of cells based on expression or a particular cell surface marker. In such separation techniques, cells to be positively selected are first contacted with specific binding agent (e.g., an antibody or reagent that interacts specifically with the cell surface marker). The cells are then contacted with retrievable particles (e.g., magnetically responsive particles) which are coupled with a reagent that binds the
- 10 specific binding agent (that has bound to the positive cells). The cell-binding agent-particle complex can then be physically separated from non-labeled cells, for example using a magnetic field. When using magnetically responsive particles, the labeled cells can be retained in a container using a magnetic field while the negative cells are removed. These and similar separation procedures are known to those of ordinary
- 15 skill in the art.

Expression of the vector in the selected transduced cells can be assessed by a number of assays known to those skilled in the art. For example, Western blot or Northern analysis can be employed depending on the nature of the inserted nucleotide sequence of interest. Once expression has been established and the transformed T

20 cells have been tested for the presence of the selected synthetic expression cassette, they are ready for infusion into a patient via the peripheral blood stream.

The invention includes a kit for genetic modification of an *ex vivo* population of primary mammalian cells. The kit typically contains a gene transfer vector coding for at least one selectable marker and at least one synthetic expression cassette

25 contained in one or more containers, ancillary reagents or hardware, and instructions for use of the kit.

#### EXPERIMENTAL

Below are examples of specific embodiments for carrying out the present

30 invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

5

### Example 1

#### Generation of Synthetic Expression Cassettes

A. Modification of HIV-1 Env, Gag, Gag-protease and Gag-polymerase Nucleic Acid Coding Sequences

The Gag, Gag-protease, and Gag-polymerase coding sequences were selected 10 from the Type C strains AF110965 and AF110967. The Env coding sequences were selected from Type C strains AF110968 and AF110975. These sequences were manipulated to maximize expression of their gene products.

First, the HIV-1 codon usage pattern was modified so that the resulting nucleic acid coding sequence was comparable to codon usage found in highly expressed 15 human genes. The HIV codon usage reflects a high content of the nucleotides A or T of the codon-triplet. The effect of the HIV-1 codon usage is a high AT content in the DNA sequence that results in a decreased translation ability and instability of the mRNA. In comparison, highly expressed human codons prefer the nucleotides G or C. The coding sequences were modified to be comparable to codon usage found in 20 highly expressed human genes.

Second, there are inhibitory (or instability) elements (INS) located within the coding sequences of the Gag and Gag-protease coding sequences (Schneider R, et al., *J Virol.* 71(7):4892-4903, 1997). RRE is a secondary RNA structure that interacts with the HIV encoded Rev-protein to overcome the expression down-regulating 25 effects of the INS. To overcome the post-transcriptional activating mechanisms of RRE and Rev, the instability elements are inactivated by introducing multiple point mutations that do not alter the reading frame of the encoded proteins. Figures 5 and 6 (SEQ ID Nos: 3, 4, 20 and 21) show the location of some remaining INS in synthetic sequences derived from strains AF110965 and AF110967. The changes made to these 30 sequences are boxed in the Figures. In Figures 5 and 6, the top line depicts a codon optimized sequence of Gag polypeptides from the indicated strains. The nucleotide(s) appearing below the line in the boxed region(s) depicts changes made to further

remove INS. Thus, when the changes indicated in the boxed regions are made, the resulting sequences correspond to the sequences depicted in Figures 1 and 2, respectively.

For the Gag-protease sequence, the changes in codon usage are restricted to the regions up to the -1 frameshift and starting again at the end of the Gag reading frame. Further, inhibitory (or instability) elements (INS) located within the coding sequences of the Gag-protease polypeptide coding sequence are altered as well. The synthetic coding sequences are assembled by methods known in the art, for example by companies such as the Midland Certified Reagent Company (Midland, Texas).

Modification of the Gag-polymerase sequences include similar modifications as described for Gag-protease in order to preserve the frameshift region.

In one embodiment of the invention, the full length polymerase coding region of the Gag-polymerase sequence is included with the synthetic Gag or Env sequences in order to increase the number of epitopes for virus-like particles expressed by the synthetic, optimized Gag/Env expression cassette. Because synthetic HIV-1 Gag-polymerase expresses the functional enzymes reverse transcriptase (RT) and integrase (INT) (in addition to the structural proteins and protease), it is important to inactivate RT and INT functions. Several deletions or mutations in the RT and INT coding regions can be made to achieve catalytic nonfunctional enzymes with respect to their RT and INT activity. {Jay. A. Levy (Editor) (1995) *The Retroviridae*, Plenum Press, New York. ISBN 0-306-45033X. Pages 215-20; Grimson, B. and Laurence, J. (1995), *Journal Of Acquired Immune Deficiency Syndromes and Human Retrovirology* 9(1):58-68; Wakefield, J. K., et al., (1992) *Journal Of Virology* 66(11):6806-6812; Esnouf, R., et al., (1995) *Nature Structural Biology* 2(4):303-308; Maignan, S., et al., (1998) *Journal Of Molecular Biology* 282(2):359-368; Katz, R. A. and Skalka, A. M. (1994) *Annual Review Of Biochemistry* 73 (1994); Jacobo-Molina, A., et al., (1993) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 90(13):6320-6324; Hickman, A. B., et al., (1994) *Journal Of Biological Chemistry* 269(46):29279-29287; Goldgur, Y., et al., (1998) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 95(16):9150-9154; Goette, M., et al., (1998) *Journal Of Biological Chemistry* 273(17):10139-10146; Gorton, J. L., et al., (1998) *Journal of Virology* 72(6):5046-5055; Engelman,

A., et al., (1997) *Journal Of Virology* 71(5):3507-3514; Dyda, F., et al., *Science* 266(5193):1981-1986; Davies, J. F., et al., (1991) *Science* 252(5002):88-95; Bujacz, G., et al., (1996) *Fefs Letters* 398(2-3):175-178; Beard, W. A., et al., (1996) *Journal Of Biological Chemistry* 271(21):12213-12220; Kohlstaedt, L. A., et al., (1992) *Science* 256(5065):1783-1790; Krug, M. S. and Berger, S. L. (1991) *Biochemistry* 30(44):10614-10623; Mazumder, A., et al., (1996) *Molecular Pharmacology* 49(4):621-628; Palaniappan, C., et al., (1997) *Journal Of Biological Chemistry* 272(17):11157-11164; Rodgers, D. W., et al., (1995) *Proceedings Of the National Academy Of Sciences Of the United States Of America* 92(4):1222-1226; Sheng, N. and Dennis, D. (1993) *Biochemistry* 32(18):4938-4942; Spence, R. A., et al., (1995) *Science* 267(5200):988-993.)

Furthermore selected B- and/or T-cell epitopes can be added to the Gag-polymerase constructs within the deletions of the RT- and INT-coding sequence to replace and augment any epitopes deleted by the functional modifications of RT and INT. Alternately, selected B- and T-cell epitopes (including CTL epitopes) from RT and INT can be included in a minimal VLP formed by expression of the synthetic Gag or synthetic GagProt cassette, described above. (For descriptions of known HIV B- and T-cell epitopes see, HIV Molecular Immunology Database CTL Search Interface; Los Alamos Sequence Compendia, 1987-1997; Internet address: <http://hiv-web.lanl.gov/immunology/index.html>.)

The resulting modified coding sequences are presented as a synthetic Env expression cassette; a synthetic Gag expression cassette; a synthetic Gag-protease expression cassette; and a synthetic Gag-polymerase expression cassette. A common Gag region (Gag-common) extends from nucleotide position 844 to position 903 (SEQ ID NO:1), relative to AF110965 (or from approximately amino acid residues 282 to 301 of SEQ ID NO:17) and from nucleotide position 841 to position 900 (SEQ ID NO:2), relative to AF110967 (or from approximately amino acid residues 281 to 300 of SEQ ID NO:22). A common Env region (Env-common) extends from nucleotide position 1213 to position 1353 (SEQ ID NO:5) and amino acid positions 405 to 451 of SEQ ID NO:23, relative to AF110968 and from nucleotide position 1210 to position 1353 (SEQ ID NO:11) and amino acid positions 404-451 (SEQ ID NO:24), relative to AF110975.

The synthetic DNA fragments for Gag and Env are cloned into the following eucaryotic expression vectors: pCMVKm2, for transient expression assays and DNA immunization studies, the pCMVKm2 vector is derived from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) **19**:3979-3986) and comprises a kanamycin selectable marker, a ColE1 origin of replication, a CMV promoter enhancer and Intron A, followed by an insertion site for the synthetic sequences described below followed by a polyadenylation signal derived from bovine growth hormone -- the pCMVKm2 vector differs from the pCMV-link vector only in that a polylinker site is inserted into pCMVKm2 to generate pCMV-link; pESN2dhfr and pCMVPLEdhfr, for expression in Chinese Hamster Ovary (CHO) cells; and, pAcC13, a shuttle vector for use in the Baculovirus expression system (pAcC13, is derived from pAcC12 which is described by Munemitsu S., et al., *Mol Cell Biol.* **10**(11):5977-5982, 1990).

Briefly, construction of pCMVPLEdhfr was as follows.

To construct a DHFR cassette, the EMCV IRES (internal ribosome entry site) leader was PCR-amplified from pCite-4a+ (Novagen, Inc., Milwaukee, WI) and inserted into pET-23d (Novagen, Inc., Milwaukee, WI) as an *Xba*-*Nco* fragment to give pET-EMCV. The *dhfr* gene was PCR-amplified from pESN2dhfr to give a product with a Gly-Gly-Gly-Ser spacer in place of the translation stop codon and inserted as an *Nco*-*Bam*H1 fragment to give pET-E-DHFR. Next, the attenuated *neo* gene was PCR amplified from a pSV2Neo (Clontech, Palo Alto, CA) derivative and inserted into the unique *Bam*H1 site of pET-E-DHFR to give pET-E-DHFR/*Neo*<sub>(m2)</sub>. Finally the bovine growth hormone terminator from pCDNA3 (Invitrogen, Inc., Carlsbad, CA) was inserted downstream of the *neo* gene to give pET-E-DHFR/*Neo*<sub>(m2)</sub>BGHt. The EMCV-*dhfr*/*neo* selectable marker cassette fragment was prepared by cleavage of pET-E-DHFR/*Neo*<sub>(m2)</sub>BGHt.

The CMV enhancer/promoter plus Intron A was transferred from pCMV6a (Chapman et al., *Nuc. Acids Res.* (1991) **19**:3979-3986) as a *Hind*III-*Sal*I fragment into pUC19 (New England Biolabs, Inc., Beverly, MA). The vector backbone of pUC19 was deleted from the *Nde*1 to the *Sap*1 sites. The above described DHFR cassette was added to the construct such that the EMCV IRES followed the CMV promoter. The vector also contained an *amp*<sup>r</sup> gene and an SV40 origin of replication.

B. Defining of the Major Homology Region (MHR) of HIV-1 p55Gag

The Major Homology Region (MHR) of HIV-1 p55 (Gag) is located in the p24-CA sequence of Gag. It is a conserved stretch of approximately 20 amino acids. The position in the wild type AF110965 Gag protein is from 282-301 (SEQ ID NO:25) and spans a region from 844-903 (SEQ ID NO:26) for the Gag DNA-sequence. The position in the synthetic Gag protein is also from 282-301 (SEQ ID NO:25) and spans a region from 844-903 (SEQ ID NO:1) for the synthetic Gag DNA-sequence. The position in the wild type and synthetic AF110967 Gag protein is from 281-300 (SEQ ID NO:27) and spans a region from 841-900 (SEQ ID NO:2) for the modified Gag DNA-sequence. Mutations or deletions in the MHR can severely impair particle production (Borsetti, A., et al., *J. Virol.* 72(11):9313-9317, 1998; Mammano, F., et al., *J Virol* 68(8):4927-4936, 1994).

Percent identity to this sequence can be determined, for example, using the Smith-Waterman search algorithm (Time Logic, Incline Village, NV), with the following exemplary parameters: weight matrix = nuc4x4hb; gap opening penalty = 20, gap extension penalty = 5.

C. Defining of the Common Sequence Region of HIV-1 Env

The common sequence region (CSR) of HIV-1 Env is located in the C4 sequence of Env. It is a conserved stretch of approximately 47 amino acids. The position in the wild type and synthetic AF110968 Env protein is from approximately amino acid residue 405 to 451 (SEQ ID NO:28) and spans a region from 1213 to 1353 (SEQ ID NO:5) for the Env DNA-sequence. The position in the wild type and synthetic AF110975 Env protein is from approximately amino acid residue 404 to 451 (SEQ ID NO:29) and spans a region from 1210 to 1353 (SEQ ID NO:11) for the Env DNA-sequence.

Percent identity to this sequence can be determined, for example, using the Smith-Waterman search algorithm (Time Logic, Incline Village, NV), with the following exemplary parameters: weight matrix = nuc4x4hb; gap opening penalty = 20, gap extension penalty = 5.

Various forms of the different embodiments of the invention, described herein, may be combined.

Example 2Expression Assays for the Synthetic Coding SequencesA. Env, Gag and Gag-Protease Coding Sequences

5       The wild-type Env (from AF110968 or AF110975), Gag (from AF110965 and AF110967) and Gag-protease (from AF110965 and AF110967) sequences are cloned into expression vectors having the same features as the vectors into which the synthetic Env, Gag and Gag-protease sequences are cloned.

10      Expression efficiencies for various vectors carrying the wild-type and synthetic Env and Gag sequences are evaluated as follows. Cells from several mammalian cell lines (293, RD, COS-7, and CHO; all obtained from the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209) are transfected with 2 µg of DNA in transfection reagent LT1 (PanVera Corporation, 545 Science Dr., Madison, WI). The cells are incubated for 5 hours in reduced serum  
15     medium (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The medium is then replaced with normal medium as follows: 293 cells, IMDM, 10% fetal calf serum, 2% glutamine (BioWhittaker, Walkersville, MD); RD and COS-7 cells, D-MEM, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD); and CHO cells, Ham's F-12, 10% fetal calf serum, 2% glutamine (Opti-MEM, Gibco-BRL, Gaithersburg, MD). The cells are incubated for either 48 or 60 hours. Cell lysates are collected as described below in Example 3. Supernatants are harvested and filtered through 0.45 µm syringe filters. Supernatants are evaluated using the Coulter p24-assay (Coulter Corporation, Hialeah, FL, US), using 96-well plates coated with a murine monoclonal antibody directed against HIV core antigen. The HIV-1 p24  
20     antigen binds to the coated wells. Biotinylated antibodies against HIV recognize the bound p24 antigen. Conjugated strepavidin-horseradish peroxidase reacts with the biotin. Color develops from the reaction of peroxidase with TMB substrate. The reaction is terminated by addition of 4N H<sub>2</sub>SO<sub>4</sub>. The intensity of the color is directly proportional to the amount of HIV p24 antigen in a sample.

25

30      Synthetic Env, Gag and Gag-protease expression cassettes provides dramatic increases in production of their protein products, relative to the native (wild-type Type C) sequences, when expressed in a variety of cell lines.

Example 3Western Blot Analysis of ExpressionA. Env, Gag and Gag-Protease Coding Sequences

5 Human 293 cells are transfected as described in Example 2 with pCMV6a-based vectors containing native or synthetic Env or Gag expression cassettes. Cells are cultivated for 60 hours post-transfection. Supernatants are prepared as described. Cell lysates are prepared as follows. The cells are washed once with phosphate-buffered saline, lysed with detergent [1% NP40 (Sigma Chemical Co., St. Louis, MO) 10 in 0.1 M Tris-HCl, pH 7.5], and the lysate transferred into fresh tubes. SDS-polyacrylamide gels (pre-cast 8-16%; Novex, San Diego, CA) are loaded with 20 µl of supernatant or 12.5 µl of cell lysate. A protein standard is also loaded (5 µl, broad size range standard; BioRad Laboratories, Hercules, CA). Electrophoresis is carried out and the proteins are transferred using a BioRad Transfer Chamber (BioRad 15 Laboratories, Hercules, CA) to Immobilon P membranes (Millipore Corp., Bedford, MA) using the transfer buffer recommended by the manufacturer (Millipore), where the transfer is performed at 100 volts for 90 minutes. The membranes are exposed to HIV-1-positive human patient serum and immunostained using o-phenylenediamine dihydrochloride (OPD; Sigma).

20 Immunoblotting analysis shows that cells containing the synthetic Env or Gag expression cassette produce the expected protein at higher per-cell concentrations than cells containing the native expression cassette. The proteins are seen in both cell lysates and supernatants. The levels of production are significantly higher in cell supernatants for cells transfected with the synthetic expression cassettes of the present 25 invention.

In addition, supernatants from the transfected 293 cells are fractionated on sucrose gradients. Aliquots of the supernatant are transferred to Polyclear™ ultracentrifuge tubes (Beckman Instruments, Columbia, MD), under-laid with a solution of 20% (wt/wt) sucrose, and subjected to 2 hours centrifugation at 28,000 rpm in a 30 Beckman SW28 rotor. The resulting pellet is suspended in PBS and layered onto a 20-60% (wt/wt) sucrose gradient and subjected to 2 hours centrifugation at 40,000 rpm in a Beckman SW41ti rotor.

The gradient is then fractionated into approximately 10 x 1 ml aliquots (starting at the top, 20%-end, of the gradient). Samples are taken from fractions 1-9 and are electrophoresed on 8-16% SDS polyacrylamide gels. The supernatants from 293/synthetic Env or Gag cells give much stronger bands than supernatants from  
5 293/native Env or Gag cells.

Example 4

In Vivo Immunogenicity of Synthetic Gag and Env Expression Cassettes

A. Immunization

10 To evaluate the possibly improved immunogenicity of the synthetic Gag and Env expression cassettes, a mouse study is performed. The plasmid DNA, pCMVKM2 carrying the synthetic Gag expression cassette, is diluted to the following final concentrations in a total injection volume of 100 µl: 20 µg, 2 µg, 0.2 µg, 0.02 and 0.002 µg. To overcome possible negative dilution effects of the diluted DNA, the  
15 total DNA concentration in each sample is brought up to 20 µg using the vector (pCMVKM2) alone. As a control, plasmid DNA of the native Gag expression cassette is handled in the same manner. Twelve groups of four to ten Balb/c mice (Charles River, Boston, MA) are intramuscularly immunized (50 µl per leg, intramuscular injection into the *tibialis anterior*) according to the schedule in Table 1.

Table 1

Group	Gag or Env Expression Cassette	Concentration of Gag or Env plasmid DNA (µg)	Immunized at time (weeks):
1	Synthetic	20	0 <sup>1</sup> , 4
2	Synthetic	2	0, 4
5	Synthetic	0.2	0, 4
4	Synthetic	0.02	0, 4
5	Synthetic	0.002	0, 4
6	Synthetic	20	0
7	Synthetic	2	0
10	Synthetic	0.2	0
8	Synthetic	0.02	0
9	Synthetic	0.002	0
10	Synthetic	20	0, 4
11	Native	2	0, 4
12	Native	0.2	0, 4
15	Native	0.02	0, 4
13	Native	0.002	0, 4
14	Native	20	0
15	Native	2	0
16	Native	0.2	0
20	Native	0.02	0
17	Native	0.002	0
18	Native	20	0
19	Native	2	0
20	Native	0.2	0
			0

1 = initial immunization at "week 0"

25 Groups 1-5 and 11-15 are bled at week 0 (before immunization), week 4, week 6, week 8, and week 12. Groups 6-20 and 16-20 are bled at week 0 (before immunization) and at week 4.

B. Humoral Immune Response

The humoral immune response is checked with an anti-HIV Gag or Env antibody ELISAs (enzyme-linked immunosorbent assays) of the mice sera 0 and 4 weeks post immunization (groups 5-12) and, in addition, 6 and 8 weeks post 5 immunization, respectively, 2 and 4 weeks post second immunization (groups 1-4).

The antibody titers of the sera are determined by anti-Gag or anti-Env antibody ELISA. Briefly, sera from immunized mice are screened for antibodies directed against the HIV p55 Gag protein or an Env protein, e.g., gp160 or gp120. ELISA microtiter plates are coated with 0.2 µg of Gag or Env protein per well 10 overnight and washed four times; subsequently, blocking is done with PBS-0.2% Tween (Sigma) for 2 hours. After removal of the blocking solution, 100 µl of diluted mouse serum is added. Sera are tested at 1/25 dilutions and by serial 3-fold dilutions, thereafter. Microtiter plates are washed four times and incubated with a secondary, peroxidase-coupled anti-mouse IgG antibody (Pierce, Rockford, IL). ELISA plates 15 are washed and 100 µl of 3, 3', 5, 5'-tetramethyl benzidine (TMB; Pierce) is added per well. The optical density of each well is measured after 15 minutes. The titers reported are the reciprocal of the dilution of serum that gave a half-maximum optical density (O.D.).

Synthetic expression cassettes will provide a clear improvement of 20 immunogenicity relative to the native expression cassettes.

C. Cellular Immune Response

The frequency of specific cytotoxic T-lymphocytes (CTL) is evaluated by a standard chromium release assay of peptide pulsed Balb/c mouse CD4 cells. Gag or 25 Env expressing vaccinia virus infected CD-8 cells are used as a positive control. Briefly, spleen cells (Effector cells, E) are obtained from the BALB/c mice immunized as described above are cultured, restimulated, and assayed for CTL activity against Gag peptide-pulsed target cells as described (Doe, B., and Walker, C.M., *AIDS* 10(7):793-794, 1996). Cytotoxic activity is measured in a standard <sup>51</sup>Cr release assay. Target (T) cells are cultured with effector (E) cells at various E:T ratios 30 for 4 hours and the average cpm from duplicate wells are used to calculate percent specific <sup>51</sup>Cr release.

Cytotoxic T-cell (CTL) activity is measured in splenocytes recovered from the mice immunized with HIV Gag or Env DNA. Effector cells from the Gag or Env DNA-immunized animals exhibit specific lysis of Gag or Env peptide-pulsed SV-BALB (MHC matched) targets cells, indicative of a CTL response. Target cells that 5 are peptide-pulsed and derived from an MHC-unmatched mouse strain (MC57) are not lysed.

Thus, synthetic Env and Gag expression cassettes exhibit increased potency for induction of cytotoxic T-lymphocyte (CTL) responses by DNA immunization.

10

Example 5

DNA-immunization of Non-Human Primates Using a Synthetic Env or Gag Expression Cassette

Non-human primates are immunized multiple times (e.g., weeks 0, 4, 8 and 24) intradermally, mucosally or bilaterally, intramuscular, into the quadriceps using 15 various doses (e.g., 1-5 mg) synthetic Gag- and/or Env-containing plasmids. The animals are bled two weeks after each immunization and ELISA is performed with isolated plasma. The ELISA is performed essentially as described in Example 4 except the second antibody-conjugate is an anti-human IgG, g-chain specific, peroxidase conjugate (Sigma Chemical Co., St. Louis, MD 63178) used at a dilution 20 of 1:500. Fifty µg/ml yeast extract is added to the dilutions of plasma samples and antibody conjugate to reduce non-specific background due to preexisting yeast antibodies in the non-human primates.

Further, lymphoproliferative responses to antigen can also be evaluated post-immunization, indicative of induction of T-helper cell functions.

25 Both synthetic Env and Gag plasmid DNA is expected to be immunogenic in non-human primates.

Example 6

In vitro expression of recombinant Sindbis RNA and DNA containing the synthetic 30 Env and Gag expression cassette

To evaluate the expression efficiency of the synthetic Env and Gag expression cassette in Alphavirus vectors, the selected synthetic expression cassette is

subcloned into both plasmid DNA-based and recombinant vector particle-based Sindbis virus vectors. Specifically, a cDNA vector construct for *in vitro* transcription of Sindbis virus RNA vector replicons (pRSIN-luc; Dubensky, et al., *J Virol.* 70:508-519, 1996) is modified to contain a *PmeI* site for plasmid linearization and a 5 polylinker for insertion of heterologous genes. A polylinker is generated using two oligonucleotides that contain the sites *XhoI*, *PmlI*, *Apal*, *NarI*, *XbaI*, and *NotI* (XPANXNF, and XPANXNR).

The plasmid pRSIN-luc (Dubensky et al., *supra*) is digested with *XhoI* and *NotI* to remove the luciferase gene insert, blunt-ended using Klenow and dNTPs, and 10 purified from an agarose gel using GeneCleanII (Biol01, Vista, CA). The oligonucleotides are annealed to each other and ligated into the plasmid. The resulting construct is digested with *NotI* and *SacI* to remove the minimal Sindbis 3'-end sequence and A<sub>40</sub> tract, and ligated with an approximately 0.4 kbp fragment from PKSSIN1-BV (WO 97/38087). This 0.4 kbp fragment is obtained by digestion of 15 pKSSIN1-BV with *NotI* and *SacI*, and purification after size fractionation from an agarose gel. The fragment contains the complete Sindbis virus 3'-end, an A<sub>40</sub> tract and a *PmeI* site for linearization. This new vector construct is designated SINBVE.

The synthetic HIV Gag and Env coding sequences are obtained from the parental plasmid by digestion with *EcoRI*, blunt-ending with Klenow and dNTPs, 20 purification with GeneCleanII, digestion with *SalI*, size fractionation on an agarose gel, and purification from the agarose gel using GeneCleanII. The synthetic Gag or Env coding fragment is ligated into the SINBVE vector that is digested with *XhoI* and *PmlI*. The resulting vector is purified using GeneCleanII and is designated SINBVGag. Vector RNA replicons may be transcribed *in vitro* (Dubensky et al., 25 *supra*) from SINBVGag and used directly for transfection of cells. Alternatively, the replicons may be packaged into recombinant vector particles by co-transfection with defective helper RNAs or using an alphavirus packaging cell line.

The DNA-based Sindbis virus vector pDCMVSIN-beta-gal (Dubensky, et al., *J Virol.* 70:508-519, 1996) is digested with *SalI* and *XbaI*, to remove the beta-30 galactosidase gene insert, and purified using GeneCleanII after agarose gel size fractionation. The HIV Gag or Env gene is inserted into the pDCMVSIN-beta-gal by digestion of SINBVGag with *SalI* and *XhoI*, purification using GeneCleanII of the

Gag-containing fragment after agarose gel size fractionation, and ligation. The resulting construct is designated pDSIN-Gag, and may be used directly for *in vivo* administration or formulated using any of the methods described herein.

BHK and 293 cells are transfected with recombinant Sindbis RNA and DNA,  
5 respectively. The supernatants and cell lysates are tested with the Coulter capture ELISA (Example 2).

BHK cells are transfected by electroporation with recombinant Sindbis RNA.  
293 cells are transfected using LT-1 (Example 2) with recombinant Sindbis DNA. Synthetic Gag- and/or Env-containing plasmids are used as positive controls.  
10 Supernatants and lysates are collected 48h post transfection.

Gag and Env proteins can be efficiently expressed from both DNA and RNA-based Sindbis vector systems using the synthetic expression cassettes.

#### Example 7

15       In Vivo Immunogenicity of recombinant Sindbis Replicon Vectors containing  
synthetic Gag and/or Env Expression Cassettes

A.       Immunization  
To evaluate the immunogenicity of recombinant synthetic Gag and Env expression cassettes in Sindbis replicons, a mouse study is performed. The Sindbis virus DNA vector carrying the synthetic Gag and/or Env expression cassette (Example 6), is diluted to the following final concentrations in a total injection volume of 100 µl: 20 µg, 2 µg, 0.2 µg, 0.02 and 0.002 µg. To overcome possible negative dilution effects of the diluted DNA, the total DNA concentration in each sample is brought up to 20 µg using the Sindbis replicon vector DNA alone. Twelve  
20 groups of four to ten Balb/c mice (Charles River, Boston, MA) are intramuscularly immunized (50 µl per leg, intramuscular injection into the *tibialis anterior*) according to the schedule in Table 2. Alternatively, Sindbis viral particles are prepared at the following doses: 10<sup>3</sup> pfu, 10<sup>5</sup> pfu and 10<sup>7</sup> pfu in 100 µl, as shown in Table 3. Sindbis Env or Gag particle preparations are administered to mice using intramuscular and  
25 subcutaneous routes (50 µl per site).  
30

Table 2

Group	Gag or Env Expression Cassette	Concentration of Gag or Env DNA ( $\mu$ g)	Immunized at time (weeks):
1	Synthetic	20	0 <sup>1</sup> , 4
2	Synthetic	2	0, 4
3	Synthetic	0.2	0, 4
4	Synthetic	0.02	0, 4
5	Synthetic	0.002	0, 4
6	Synthetic	20	0
7	Synthetic	2	0
8	Synthetic	0.2	0
9	Synthetic	0.02	0
10	Synthetic	0.002	0

1 = initial immunization at "week 0"

Table 3

Group	Gag or Env sequence	Concentration of viral particle (pfu)	Immunized at time (weeks):
1	Synthetic	10 <sup>3</sup>	0 <sup>1</sup> , 4
2	Synthetic	10 <sup>5</sup>	0, 4
3	Synthetic	10 <sup>7</sup>	0, 4
8	Synthetic	10 <sup>3</sup>	0
9	Synthetic	10 <sup>5</sup>	0
10	Synthetic	10 <sup>7</sup>	0

1 = initial immunization at "week 0"

Groups are bled and assessment of both humoral and cellular (e.g., frequency  
25 of specific CTLs) is performed, essentially as described in Example 4.

Although preferred embodiments of the subject invention have been described in some detail, it is understood that obvious variations can be made without departing from the spirit and the scope of the invention as defined by the appended claims.

CLAIMS

1. An expression cassette, comprising  
a polynucleotide sequence encoding a polypeptide including an HIV *Gag* polypeptide, wherein the polynucleotide sequence encoding said *Gag* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as either nucleotides 844-903 of Figure 1 (SEQ ID NO:1) or nucleotides 841-900 of Figure 2 (SEQ ID NO:2).
- 10 2. An expression cassette, comprising  
a polynucleotide sequence encoding a polypeptide including an HIV *Gag* polypeptide, wherein the polynucleotide sequence encoding said *Gag* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as Figure 1 (SEQ ID NO:3) or Figure 2 (SEQ ID NO:4).
- 15 3. The expression cassette of claim 2, wherein said polynucleotide sequence encoding a polypeptide including an HIV *Gag* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as Figure 1 (SEQ ID NO:3).
- 20 4. The expression cassette of claim 2, wherein said polynucleotide sequence encoding a polypeptide including an HIV *Gag* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as Figure 2 (SEQ ID NO:4).
- 25 5. The expression cassette of claim 2, wherein the polynucleotide sequence encoding said *Gag* polypeptide consists of a sequence having the sequence presented as Figure 1 (SEQ ID NO:3).
- 30 6. The expression cassette of claim 2, wherein the polynucleotide sequence encoding said *Gag* polypeptide consists of a sequence having the sequence presented as Figure 2 (SEQ ID NO:4).

7. The expression cassette of any of claims 1 to 6, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *protease* polypeptide.

5

8. The expression cassette of any of claims 1 to 6, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *polymerase* polypeptide.

10

9. The expression cassette of any of claims 1 to 6, wherein said polynucleotide sequence further includes a polynucleotide sequence encoding an HIV *polymerase* polypeptide, wherein the sequence encoding the HIV *polymerase* polypeptide is modified by deletions of coding regions corresponding to reverse transcriptase and integrase.

15

10. The expression cassette of claim 9, wherein said polynucleotide sequence preserves T-helper cell and CTL epitopes.

20

11. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1213-1353 of Figure 3 (SEQ ID NO:5).

25

12. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 82-1512 of Figure 3 (SEQ ID NO:6).

30

13. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 82-2025 of Figure 3

(SEQ ID NO:7).

14. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 5 90% sequence identity to the sequence presented as nucleotides 82-2547 of Figure 3 (SEQ ID NO:8).

15. The expression cassette of claim 11, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 10 90% sequence identity to the sequence presented as nucleotides 1-2547 of Figure 3 (SEQ ID NO:9).

16. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the 15 polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1513-2547 of Figure 3 (SEQ ID NO:10).

17. An expression cassette, comprising a polynucleotide sequence 20 encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1210-1353 of Figure 4 (SEQ ID NO:11).

25 18. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 73-1509 of Figure 4 (SEQ ID NO:12).

30 19. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 73-2022 of Figure 4

(SEQ ID NO:13).

20. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 5 90% sequence identity to the sequence presented as nucleotides 73-2565 of Figure 4 (SEQ ID NO:14).

21. The expression cassette of claim 17, wherein the polynucleotide sequence encoding said *Env* polypeptide further comprises a sequence having at least 10 90% sequence identity to the sequence presented as nucleotides 1-2565 of Figure 4 (SEQ ID NO:15).

22. An expression cassette, comprising a polynucleotide sequence encoding a polypeptide including an HIV *Env* polypeptide, wherein the 15 polynucleotide sequence encoding said *Env* polypeptide comprises a sequence having at least 90% sequence identity to the sequence presented as nucleotides 1510-2565 of Figure 4 (SEQ ID NO:16).

23. An expression cassette, comprising a polynucleotide sequence 20 encoding a polypeptide including an HIV *Env* polypeptide, wherein the polynucleotide sequence encoding said *Env* polypeptide consists of a sequence having the sequence presented as Figure 3 (SEQ ID NO:9) or Figure 4 (SEQ ID NO:15).

24. A recombinant expression system for use in a selected host cell, 25 comprising, an expression cassette of any of claims 1-23, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected host cell.

25. The recombinant expression system of claim 24, wherein said control 30 elements are selected from the group consisting of a transcription promoter, a transcription enhancer element, a transcription termination signal, polyadenylation sequences, sequences for optimization of initiation of translation, and translation

termination sequences.

26. The recombinant expression system of claim 24, wherein said transcription promoter is selected from the group consisting of CMV, CMV+intron A,  
5 SV40, RSV, HIV-Ltr, MMLV-ltr, and metallothionein.

27. A cell comprising an expression cassette of any of claims 1-23, and wherein said polynucleotide sequence is operably linked to control elements compatible with expression in the selected cell.

10

28. The cell of claim 27, wherein the cell is a mammalian cell.

29. The cell of claim 28, wherein the cell is selected from the group consisting of BHK, VERO, HT1080, 293, RD, COS-7, and CHO cells.

15

30. The cell of claim 29, wherein said cell is a CHO cell.

31. The cell of claim 27, wherein the cell is an insect cell.

20

32. The cell of claim 31, wherein the cell is either *Trichoplusia ni* (Tn5) or Sf9 insect cells.

33. The cell of claim 27, wherein the cell is a bacterial cell.

25

34. The cell of claim 27, wherein the cell is a yeast cell.

35. The cell of claim 27, wherein the cell is a plant cell.

36. The cell of claim 27, wherein the cell is an antigen presenting cell.

30

37. The cell of claim 36, wherein the lymphoid cell is selected from the group consisting of macrophage, monocytes, dendritic cells, B-cells, T-cells, stem cells, and

progenitor cells thereof.

38. The cell of claim 27, wherein the cell is a primary cell.
- 5        39. The cell of claim 27, wherein the cell is an immortalized cell.
40. The cell of claim 27, wherein the cell is a tumor-derived cell.
- 10      41. A composition for generating an immunological response, comprising:  
an expression cassette of any of claims 1-10.
42. The composition of claim 41, further comprising a *Gag* polypeptide  
encoded by at least one of the expression cassettes of any of claims 1-10.
- 15      43. The composition of claim 41, further comprising an adjuvant.
44. A composition for generating an immunological response, comprising:  
an expression cassette of any of claims 11-23.
- 20      45. The composition of claim 44, further comprising an *Env* polypeptide  
encoded by at least one of the expression cassettes of any of claims 11-23.
46. The composition of either claim 44 or 45 further comprising:  
an expression cassette of any of claims 1-10.
- 25      47. The composition of claim 46, further comprising the *Gag* polypeptide  
encoded by at least one expression cassette of any of claims 1-10.
48. The composition of claim 44, further comprising an adjuvant.
- 30      49. A method of immunization of a subject, comprising,  
introducing a composition of any of claims 41-48 into said subject under

conditions that are compatible with expression of said expression cassette in said subject.

50. The method of claim 49, wherein said expression cassette is introduced  
5 using a gene delivery vector.

51. The method of claim 50, wherein the gene delivery vector is a non-viral  
vector.

10 52. The method of claim 50, wherein said gene delivery vector is a viral  
vector.

53. The method of claim 52, wherein said gene delivery vector is a Sindbis-  
virus derived vector.

15 54. The method of claim 52, wherein said gene delivery vector is a retroviral  
vector.

20 55. The method of claim 52, wherein said gene delivery vector is a lentiviral  
vector.

56. The method of claim 49, wherein said composition delivered using a  
particulate carrier.

25 57. The method of claim 49, wherein said composition is coated on a gold or  
tungsten particle and said coated particle is delivered to said subject using a gene gun.

58. The method of claim 49, wherein said composition is encapsulated in a  
liposome preparation.

30 59. The method of any of claims 49-58, wherein said subject is a mammal.

60. The method of claim 59, wherein said mammal is a human.
61. A method of generating an immune response in a subject, comprising:  
providing an expression cassette of any of claims 1-23,  
expressing said polypeptide in a suitable host cell,  
isolating said polypeptide, and  
administering said polypeptide to the subject in an amount sufficient to elicit  
an immune response.  
5
62. A method of generating an immune response in a subject, comprising  
introducing into cells of said subject an expression cassette of any of claims 1-  
23, under conditions that permit the expression of said polynucleotide and production  
of said polypeptide, thereby eliciting an immunological response to said polypeptide.  
10
63. The method of claim 62, where the method further comprises  
administration of a polypeptide produced by an expression cassette according to any  
of claims 1-23 to the subject.  
15
64. The method of claim 63, wherein administration of the polypeptide to the  
subject is carried out before introducing said expression cassette.  
20
65. The method of claim 63, wherein administration of the polypeptide to the  
subject is carried out concurrently with introducing said expression cassette.
66. The method of claim 63, wherein administration of the polypeptide to the  
subject is carried out after introducing said expression cassette.  
25

**Gag\_AF110965\_BW\_mod**

ATGGGCGCCCGGCCAGCATCCTGCGCGGCGGCAAGCTGGACGCCTGGGAGCGCATCCGCC  
TGCGCCCGGCCGGCAAGAAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGCGAGCT  
GGAGAAGTTCCGCCCTGAACCCGGCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATC  
CGCCAGCTGCACCCGCCCTGCAGACCGGCAGCGAGGAGCTGAAGAGCCTGTTAACACCG  
TGGCCACCCCTGACTGCGTGCACGAGAAGATCGAGGTCGCGACACCAAGGAGGCCCTGGA  
CAAGATCGAGGAGGAGCAGAACAAAGTGCCAGCAGAAGATCCAGCAGGCCAGGCCGAC  
AAGGGCAAGGTGAGCCAGAACTACCCCCTGCAAGAACCTGCAGGGCCAGATGGTGCACC  
AGGCATCAGCCCCCGCACCTGAACGCCCTGGGTGAAGGTGATCGAGGAGAAGGCCCTCAG  
CCCCGAGGTGATCCCCATGTTCACCGCCCTGAGCGAGGGGCCACCCCGAGGACCTGAAC  
ACGATGTTGAACACCGTGGCGGCCACCAGGCCCATGCAAGATGCTGAAGGACACCATCA  
ACGAGGAGGCCCGCAGTGGGACCGCGTGCACCCCGTGCACGCCGGCCCATGCCCGG  
CCAGATGCGCGAGCCCCCGGCCAGCGACATGCCGGCACCAAGCACCCCTGCAGGAGCAG  
ATCGCCTGGATGACCAGAACCCCCCATCCCCGTGGCGACATCTACAAGCGGTGGATCA  
TCCTGGGCTGAACAAGATCGTGC GGATGTACAGCCCCGTGAGCATCCTGGACATCAAGCA  
GGGCCCAAGGAGCCCTCCGCGACTACGTGGACCGCTTCTTCAAGACCCCTGCGCGCCGAG  
CAGAGCACCCAGGAGGTGAAGAACTGGATGACCGACACCCCTGCTGGTGAGAACGCCAAC  
CCGACTGCAAGACCATCCTGCGCGCTCGCCCCGGGCCAGCCTGGAGGAGATGATGAC  
CGCCTGCCAGGGCGTGGCGGCCAGCCACAAGGCCCGCGTGTGGCCGAGGCATGAGC  
CAGGCCAACACCAGCGTGTGATGCAGAAGAGCAACTCAAGGGCCCCGGCGCATCGTCA  
AGTGCTTCAACTGCGCAAGGAGGGCCACATGCCCGCAACTGCCCGCCCCCGCAAGAA  
GGGCTGCTGGAAGTGCGCAAGGAGGGCCACCAAGATGAAGGACTGCACCGAGGCCAGGCC  
AACTCCTGGCAAGATCTGGCCAGCCACAAGGGCCGCCGGCAACTTCCGAGGAGACCACCCCGCCA  
GAAGCAGGAGAGCAAGGACCGCGAGACCTGACCGCCTGAAGAGCCTGTTGGCAACGAC  
CCCCCTGAGCCAGTAA

**FIG. 1**

2 / 6

**Gag\_AF110967\_BW\_mod**

ATGGGCGCCCGGCCAGCATCCTGCGCGGCAGAAGCTGGACAAGTGGAGAAGATCCGCC  
TGCGCCCGGCCAGAACACTACATGCTGAAGCACCTGGTGTGGCCAGCCGCGAGCT  
GGAGGGCTTCGCCCTGAACCCCGGCCCTGCTGGAGACCAGGGCTGCAAGCAGATCATG  
AAGCAGCTGCAGCCGCCCTGCAGACCGGACCCGAGGAGCTGCGCAGCCTGTACAACACCG  
TGGCCACCCCTGTACTGCGTGCACGCCGGCATCGAGGTCCCGACACCAAGGAGGCCCTGGA  
CAAGATCGAGGAGGAGCAGAACAAAGTCCCAGCAGAAGACCCAGCAGGCCAAGGAGGCCGAC  
GGCAAGGTGAGCCAGAACTACCCCATCGTGAGAACCTGCAGGGCCAGATGGTGCACCAGG  
CCATCAGCCCCCGCACCTGAACGCCCTGGGTGAAGGTGATCGAGGAGAAGGCCCTCAGCCC  
CGAGGTGATCCCCATGTTCACGCCCTGAGCGAGGGGCCACCCCCCAGGACCTGAACACG  
ATGTTGAACACCCTGGCGGCCACCAGGCCCATGCAGATGCTGAAGGACACCATCAACG  
AGGAGGCCGCCAGTGGGACCGCCCTGCACCCGTGCAGGCCGCCGTGGCCCCCGCCA  
GATGCGCAGCCCCCGGCCAGCGACATGCCGCCACCCAGCACCCCTGCAGGAGCAGATC  
GCCTGGATGACCAGCAACCCCCCGTGCCCGTGGCGACATCTACAAGCGGTGGATCATCC  
TGGGCTGAACAAGATCGTGC GGATGTACAGCCCCGTGAGCATCCTGGACATCCGCCAGGG  
CCCCAAGGAGCCCTCCCGCACTACGTGGACCGCTTCTCAAGACCCCTGCAGGCCAGCAG  
GCCACCCAGGACGTGAAGAACTGGATGACCGAGACCCCTGCTGGTGCAGAACGCCAACCCG  
ACTGCAAGACCATCCTGCGCCTCGGCCACTACGGACCGCTTCTCAAGACCCCTGCAGGCCAG  
CTGCCAGGGCGTGGCGGCCACAAGGCCCGCTGCTGCCAGGGCGATGAGCCAG  
GCCAACAGCGTGAACATCATGATGCAGAAGAGCAACTTCAAGGGCCCCCGCGCAACGTCA  
AGTGCTTCAACTGCGCAAGGAGGGCCACATGCCAAGAACTGCCGCCCCCCCGCAAGAA  
GGGCTGCTGGAAGTGC CGCAAGGAGGGCCACCGATGAAGGACTGCACCGAGGCCAGGCC  
AACTCCTGGCAAGATCTGGCCCAAGGGCCACCGAGAGCTTCCGCTTCAAGGAGGCC  
GCAGCGAGCCCCGCCGCCACCGTGCCTGCCACCGGCCAGGCCAGGCCAGGCCAGGCC  
GGAGACCACCCCGCCCCCAAGCAGGAGGCCAAGGACCGCAGGCCCTACCGCGAGGCCAGTAA  
ACCGCCCTGCCAGCCTGTTGGCAGCGGCCAGGCCAGTAA

**FIG. 2**

**Env\_AF110968\_C\_BW\_opt**

--> signal peptide (1-81)  
ATGCCGCGTGTGGCATCCTGAAGAACTACCAGCAGTGGTGGATGTGGGCATCCTGGCTTCTGGATGCTGATCA  
\--> gp120 (140/160) (82)  
TCAGCAGCGTGTGGCAACCTGTGGTGACCGTGTACTACGGCGTCCCCGTGTGGAAAGGAGGCCAAGACCACCC  
GTTCTGCACCGCACGCCAAGGCCAACGAGACCGAGGTGCACAACTGTGTGGCCACCCACGCCCTGCCGTC  
GACCCCCAACCCCCAGGAGATCGTGTGGAGAACGTGACCGAGAACCTCAACATGTGGAAAGAACGACATGGTGGACC  
AGATGCACGAGGACATCATCAGCCTGTGGGACCAAGGCCAGAGCCTGAAGGCCCTGCCGTGAAGCTGACCCCCCTGTGCCGTC  
CCTGAAGTGGCGCAACGTGAACGCCACCAACAAACATCAACAGCATGATCGACAAACAGCAACAAGGGCAGATGAAG  
AACTGCAGCTTCAACGTGACCAACCGAGCTGCCGACCGCAAGCAGGAGGTGCACGCCCTGTTCTACCCCTGGACG  
TGGTGCCCTGCAGGGCAACAACAGCACCGAGTACCGCCTGATCAACTGCAACACCCAGGCCATACCCAGGCC  
CCCCAAGGTGAGCTTCGACCCCATCCCCATCCACTACTGCACCCCCGCCGGCTACGCCATCCTGAAGTGCACAAAC  
CAGACCTTCAACGGCACCGGCCCTGCAACACAGTGAAGCAGCTGAGCAGCGTGCAGTGCACGCCACGGCATCAAGCCC  
TGGTGAAGCTGCTGCTGAACGGCAGCCTGGCCAGGGCGAGATCATCCGAGCGAGAACCTGGCAACAACGCC  
CAAGATCATCATCGTGCAGCTGAACAAGCCCAGTGAAGATCGTGTGCCTGCCAGAACACACCCAGGCC  
GTGGCGCATGGCCCCGGCCAGACCTTCTACGCCACCGCGAGATCATCCGACATCCGCCAGGCCACTGCATCA  
TCAACAAGACCGAGTGGAACAGCACCCCTGCAGGGCGTGAAGAACGACTGGAGGAGCACCTCAGCAAGAACGCC  
CAAGTTGGAGCCCAGCAGCGCGGGGACCTGGAGATCACCAACCCACAGCTCAACTGCCCGGGGAGTTCTTCTAC  
TGGCAGACCCAGCAGCTGTTCAACAGCACCTACAGCCCAGCTTCAACGGCACCGAGAACAGCTGAACGGCACCA  
TCACCATCACCTGCCCATCAAGCAGATCATCAACATGTGGCAGAACGAGTGGCCGCGCCATGTACGCC  
CGCCGGCAACCTGACCTGCGAGAGAACATCACCGCCTGCTGCTGACCCGACGGCGCAAGACCGGCCAAC  
GACACCGAGATCTCCGCCCCGGCGCGACATGCGCAGAACACTGGCGAACGAGCTGTACAAGTACAAGGTGG  
TGGAGATCAAGCCCCGGCGTGGGCCCCACCGAGGCCAACGGCGCGTGGTGGAGCGCGAGAACGCC  
CATCGGCCGCGTGTCTGGGCTTCTGGGCCGCGCGAGCACCAGGGCGCCAGCATCACCTGACCGT  
CAGGCCCTGCTGCTGAGCGCATCGTCAGCAGCACCGAGAACACCTGCTGCCGCCATCGAGGCC  
TGCTGCAGCTGACCGTGTGGGATCAAGCAGCTGCAGACCCGATCCTGGCGTGGAGCGCTACCTGAAGGCC  
GCAGCTGCTGGCATCTGGGCTGCGAGCGCAAGCTGATCTGCACCCCGTGGCTGGAACAGCAGCTGGAGC  
AACCGCAGCCACGAGATCTGGGACAACATGACCTGGATGCAGTGGGACCGCAGAACATCAACAA  
CCATCTACCGCCTGCTGGAGGAGAGGCCAGAACCCAGCAGGAGAACGAGAACGCC  
GCAGAACCTGTGGAACTGGTTCAAGCATCACCAACTGGCTGTTGAGCTACATCAAGATCTTCA  
CTGATCGCCCTGCGCATCATCTCGCCGTGCTGAGCATCGTGAACCGCGTGCCTGGGCTACAGCCCC  
TCCAGACCCCTGACCCCAACCCCGCGAGCCCCACCGCCTGGGCCGATCGAGGAGGAGGGCG  
CGGCCGAGCATCCGCTGGTGAAGCGGCTTCTGGCCCTGGCCTGGAGACCTGCGCAGCCTGTGCC  
TACCAACCGCCTGCGCATCTGATCGCCCGCGTGTGGAGCTGCTGGGCCAGCGCGGCTGGAGGCC  
TGAAGTACCTGGGAGCCCTGGTGAAGTACTGGGCTGGAGCTGAAGAACGCC  
GCCCATCGCCGTGGCCAGGGCACCGACCGCATCATCGAGTTCACTCAGCGCATCTGCCGCC  
CCCCGCCATCCGCCAGGGCTTCGAGGCCGCCCTGCAGTAA  
gp120 (1512) <--\--> (1513) gp41  
gp140 (2025) <--\-->  
gp160, gp41 (2347) <--\-->

**FIG. 3**

**Env\_AF110975\_C\_BW\_opt**

--> signal peptide (1-72)  
 ATGCCGCGTGCAGCAGCTGGCAGCAGTGGTGGATCTGGGCACTCTGGCTTCTGGATCTGCAGCG  
 gp120/140/160 (72) *\-->*  
 GCCTGGCAACCTGTGGTGACCGTGTACGACGGCTGCCCTGTGGCCGAGGCCAGCACCCCTGTTCTGC  
 CAGCGACGCCAAGCCTACGAGAAGGAGGTGCACAACGTGTGGGCCACCCACGCCCTGCCTGCCGAC  
 CCCCAGGAGATCGAGCTGGACAACGTGACGGAGAACCTCAACATGTGGAAGAACGACATGGTGGACCAGATGC  
 AGGACATCATCAGCTGTGGGACCAAGGCTGAAGCCCCCGTGAAGCTGACCCCCCTGTGCCTGACCCCTGAAGTG  
 CACCAACTACAGCACCACACTACAGCAACACCATGAACGCCACCGAGTACAACAACAAACACCACCGAGGAGATCAAG  
 AACTGCACCTTCACATGACCACCGAGCTGCCGACAAGAACGAGCAGGTGTACGCCCTGTTCTACAAGCTGGACA  
 TCGTGCCCTGAACAGCAACAGCAGCGAGTACCGCCTGATCAACTGCAACACCAGGCCATCACCCAGGCCCTGCC  
 CAAGGTGAGCTTCGACCCCATCCCCATCCACTACTGCGCCCCCGCCGCTACGCCATCCTGAAGTGAAGAACAAAC  
 ACCAGCAACGGCACCGGCCCCCTGCCAGAACGTGAGCACCGCTGAGTCAACAGCCACGGCATCAAGCCCCGTGGTGAGCA  
 CCCCCCTGCTGCTGAACGGCAGCCTGGCGAGGGCGGAGATCATCATCCGAGCAAGAACCTGAGCAACAAACGC  
 CTACACCATCATGTCACCTGAACGACAGCGTGGAGATCGTGTGACCCGCCAACAAACAAACACCCGCAAGGGC  
 ATCCGCATCGGCCCCGGCCAGACCTTCTACGCCACCGAGAACATCATCGGCACATCGGCCAGGCCACTGCAACA  
 TCAGGCCGGCGAGTGGAAACAAGGGCGTGCAGCGCTGAGCGCCAAGCTGCGCGAGGACTTCCCCAACAGACCAT  
 CGAGTCCAGCCCAGCAGCGGGGAGCTGGAGATCACCAACCCACAGCTTCAACTGCCGGCGAGTTCTTCTAC  
 TGCAACACCAAGCAAGCTGTTCAACAGCAGCTACAACGGCACAGCTACCGGGCACCAGAGCAACAGCAGCATCA  
 TCACCCCTGCCCTGCGCATCAAGCAGATCATGACATGTGGCAGAAGGTGGCCGCCATCATGCCAGGCCAG  
 CGAGGGCAACATCACCTGCAGCAGCAGCATCACCGCCCTGCTGTGGCCGCCAGGGCCCTGGACAACATCACC  
 ACCGAGATCTTCCGCCCCCAGGGCGGAGATGAAGGACAACCTGGCGAACAGAGCTGTACAAGTACAAGGTGGTGG  
 gp120 (1509) <--\--> (1510) gp41  
 AGATCAAGCCCCCTGGCGTGGCCCCACCGAGGCAAGGCCAGCGCTGGAGCTGGAGCGAGAACGCGCCGTGGCGAT  
 CGGGCCCGTGAATCTCGGCTTCTGGCGCCGCCGGCAGAACATGGCGCCGCCAGCATCACCCCTGACCGCCAG  
 GCCCCCAGCTGCTGAGCGGATCGTGCAGCAGAGAACCTGCTGCCGCCATGAGGCCAGCAGCACATGC  
 TGCAGCTGACCGTGTGGGCATCAAGCAGCTGCAAGGCCCTGCTGGCCATCGAGCGCTACCTGAAGGACCAGCA  
 GCTGCTGGCATTGGGGCTGCAGCGCAAGCTGATCTGCCACCCACCGTGGCCATGAGGCCAGCAGCTGGAGCAAC  
 AAGACCCAGGGCGAGATCTGGGAGAACATGACCTGGATGCACTGGAGCTGAGCAACTACACCGGCATCA  
 TCTACCGCCTGCTGGAGGAGGCCAGAACAGCAGGGAGCAGAACGAGAAGGACCTGCTGGCCCTGGACAGCCGCAA  
 gp140 (2022) <--\-->  
 CAACCTGTGGAGCTGGTCAACATCAGCAACTGGCTGTGGTACATCAAGATCTTCATCATGATGTGGCGGCC  
 ATCGGCCCTGCGCATCATTCGCCGTGCTGAGCATCGTGAACCGCGTGCAGGCCAGGGCTACAGCCCCCTGAGCTTCC  
 AGACCCCTGACCCCCAACCCCCCGGGCTGGACCGCCCTGGCCGCCATCGAGGAGGAGGGCGGAGCAGGACCGCGA  
 CCGCAGCATCCGCTGGTGCAGGGCTTCTGGCCCTGGCCCTGGAGCAGCTGCCAGGCCAGCTGTGCCCTGTTCA  
 CACCGCCTGCGGCACCTGATCTGGTGCACGCCCGCTGGAGCTGCTGGCCGCCAGCAGCCCCCGCGGCC  
 AGCGCGCTGGAGGCCCTGAAGTACCTGGCAGCCTGGTGCAGTACTGGGCTGGAGCTGAAGAACAGCGCCAC  
 CAGCCTGTGGACAGCATGCCATGCCGTGCCAGGGCACCGACCGCATCGAGGGTGTCCAGCGCATCTAC  
 CGCGCCTTCTGCAACATCCCCGCCGTGCCAGGGCTTCGAGGCCGCCCTGCAGTAA

**FIG. 4**

**Gag\_AF110965\_BW\_opt**

ATGGGGCCCGCGCCAGCATCCTGCGCGCGCAAGCTGGACGCCCTGGGAGCGCATCCGCCTGCGCCCCGG  
CGGCAAGAAGTGCTACATGATGAAGCACCTGGTGTGGGCCAGCCGAGCTGGAGAAGTTGCCCTGAACC  
CCGGCCTGCTGGAGACCAGCGAGGGCTGCAAGCAGATCATCCGCCAGCTGCACCCGCCCTGCAGACCGGC  
AGCGAGGAGCTGAAGAGCCTGTTCAACACCGTGGCACCCCTGTACTGCGTGCACGAGAAGATCGAGGT[G]  
CGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGCAGAACAAAGTGCAGCAGAACAGATCCAGCAGGCC  
AGGCCGCCACAAGGGCAAGGTGAGCCAGAACTACCCCCTCGTCAGAACCTGCAGGGCAGATGGTGCAC  
CAGGCCATCAGCCCCCGCACCCCTGAACGCCCTGGGTGAAGGTGATCGAGGAGAACGCCCTCAGCCCCGAGGT  
GATCCCCATGTTCACCGCCCTGAGCGAGGGCGCACCCCGAGGACCTGAACACCATG[G]TAACACCGTGG  
GCGGCCACCAGGCCCATGCAAGATGCTGAAGGACACCATCAACGAGGAGGCCGCCAGTGGGACCGCGTG  
CACCCCGTGCACGCCGGCCCCATGCCCGGCCAGATGCGCAGGCCGCCGCCAGCGACATGCCGGCAC  
CACCAAGCACCCCTGCAGGAGCAGATGCCCTGGATGACCAGCAACCCCCCATCCCCGTGGCGACATCTACA  
AGCG[G]GGATCATCCTGGGCTGAACAAGATCGTGC[G]ATGTACAGCCCCGTGAGCATCCTGGACATCAAG  
CAGGGCCCCAAGGAGGCCCTCCCGACTACGTGGACCGCTTCAAGACCCCTGCGGCCAGCAGAGCAC  
CCAGGAGGTGAAGAAGTGGATGACCGACACCCCTGCTGGTGCAGAACGCCAACCCGACTGCAAGACCATCC  
TGC[G]GCCTGGGCCCGCGCCAGCCTGGAGGAGATGATGACCGCCTGCCAGGGGTGGCGGCCAGC  
T[C]ACAAGGCCCGCGTGTGG[G]AGGCCATGAGCCAGGCCAACACCGCGTGTATGATGCAAGAGAGCAACTT  
CAAGGGCCCCCGCGATCGT[G]AGTGCTTCAACTGCGCAAGGAGGCCACATGCCCGCAACTGCCCG  
CCCCCGCAAGAAGGGCTGCTGGAAGTGCAGCAAGGAGGCCACAGATGAAGGACTGCACCGAGGCCAG  
GCCAACTTCCCTGGCAAGATCTGGCCAGCCACAAGGGCGCCGGCAACTTCCCTGCAGAGGCCCGCA  
GCCCACCGCCCCCCCCCGCCAGAGAGCTTCCGCTTCAGGGAGACCACCCCGGCCAGAACAGCAGGAGAGCAAGG  
ACCGCGAGACCCCTGACCGCCTGAAGAGCCTGTTGGCAACGACCCCTGAGCCAGTAA

**FIG. 5**

**Gag\_AF110967\_BW\_opt**

ATGGGCGCCGCCAGCATCCTGCGCGCGAGAAGCTGGACAAGTGGAGAAGATCCGCCTGCGCCCCGG  
 CGGCAAGAACGCACTACATGCTGAAGCACCTGGTGTGGGCCAGCCGCGAGCTGGAGGGCTTCGCCCTGAACC  
 CCGGCCTGCTGGAGACCGCCGAGGGCTGCAAGCAGATCATGAAGCAGCTGCAGCCGCCCTGCAGACCGGC  
 ACCGAGGAGCTGCGCAGCCTGTACAACACCGTGGCACCCCTGTACTGCGTGCACGCCGCATCGAGGTGCG  
 CGACACCAAGGAGGCCCTGGACAAGATCGAGGAGGAGCAGAACAGAGCCAGCAGAACAGCCAGCAGGCCA  
 TC  
 AGGAGGCCGACGGCAAGGTGAGCCAGAACTACCCCATCGTGCAGAACCTGCAGGGCCAGATGGTGCACCAG  
 GCCATCAGCCCCCGCACCCCTGAACGCCTGGTGAAGGTGATCGAGGAGAACGGCTTCAGCCCCGAGGTGAT  
 CCCCATGTTACCGCCCTGAGCGAGGGGCCACCCCCCAGGACCTGAACACATGCTGAACACCGTGCG  
 G G T  
 GCCACCAGGCCCATGCAGATGCTGAAGGACACCATCAACGAGGAGGCCGAGTGGGACCGCCCTGCAC  
 CCCGTGCAGGCCGCCCGTGGCCCCGCCAGATGCGCAGCCCCCGCCGAGCAGATCGCCGGGCCAC  
 CAGCACCCCTGCAGGAGCAGATGCCTGGATGACCAGCAACCCCCCGTGCCTGGGACATCTACAAGC  
 GCTGGATCATCCTGGCCTGAACAAAGATCGTGCCTGTACAGCCCCGTGAGCATCCTGGACATCCGCCAG  
 GGCCCCAAGGAGGCCCTCCGCACTACGTGGACCGCTTCAAGACCCCTGCGCGCCAGCAGGCCACCCA  
 GGACGTGAAGAACTGGATGACCGAGACCCCTGCTGGTGCAGAACGCCAACCCGACTGCAAGACCATCCTGC  
 GCGCCCTGGCCCCGGGCCACCCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGCCCCGGCCAC  
 T C  
 AAGGCCCGGTGCTGGCGAGGCCATGAGCCAGGCCACAGCGTGAACATCATGATGCGAGAACAGCAACTT  
 CAAGGGCCCCCGCGAACGTGAAGTGCTTCAACTGCGGCAAGGAGGGCCACATGCCAACAGAACCTGCCGCG  
 G G C  
 CCCCCCGCAAGAAGGGCTGCTGGAAAGTGCAGCAAGGAGGGCCACCAAGATGAAGGACTGCACCGAGGCCAG  
 GCCAACTTCCCTGGCAAGATCTGGCCAGCCACAAGGGCGCCCCGGCAACTTCCCTGCAGAACCGCAGCGA  
 GCCCGCCGCCACCGTGCCTGCCACCGCCCCCGCCAGAGCTTCCGCTTGCAGGAGACCACCCCCGGCC  
 CCAAGCAGGAGGCCAAGGACCGCGAGCCCTACCGCAGGCCCTGACCGCCCTGCGCAGCCTGTTCGGCAGC  
 GGCCCCCTGAGCCAGTAA

**FIG. 6**

## SEQUENCE LISTING

<110> Chiron Corporation

<120> POLYNUCLEOTIDES ENCODING ANTIGENIC HIV TYPE C  
POLYPEPTIDES, POLYPEPTIDES AND USES THEREOF

<130> 1631.100

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<150> 60/152,195

<151> 1999-09-01

<160> 29

<170> PatentIn Ver. 2.0

<210> 1

<211> 60

<212> DNA

<213> Human immunodeficiency virus

<400> 1

gacatcaagc agggcccaa ggagcccttc cgcgactacg tggaccgctt cttcaagacc 60

<210> 2

<211> 60

<212> DNA

<213> Human immunodeficiency virus

<400> 2

gacatccgcc agggcccaa ggagcccttc cgcgactacg tggaccgctt cttcaagacc 60

<210> 3

<211> 1479

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Gag  
of HIV strain AF110965

<400> 3

atgggcggcc ggcgcaggcat cctgcgcggc ggcaagctgg acgcctggga ggcgcattccgc 60  
ctgcgcggcc gggcaagaa gtgtacatg atgaagcacc tgggtgtgggc cagccgcgag 120  
ctggagaagt tcgcctgaa cccccggctg ctggagacca gcgaggggctg caagcagatc 180  
atccgcgcgc tgacacccgc cctgcagacc ggcagcggagg agctgaagag cctgttcaac 240  
accgtggcca ccctgtactg cgtgcacgag aagatcgagg tccgcgcacac caaggaggcc 300  
ctggacaaga tcgaggagga gcagaacaag tgccagcaga agatccagca ggccgaggcc 360  
gcccacaagg gcaagggttag ccagaactac cccatcgtgc agaacctgca gggccagatg 420  
gtgcacccagg ccatcagccc cccgcacccctg aacgcctggg tgaagggtat cgaggagaag 480  
gccttcagcc cggaggtat ccccatgttc accgcctgaa gcgaggggcgc caccggcc 540  
gacctgaaaca cgatgttgaa caccgtgggc ggccacccagg cccgcattgca gatgctgaag 600  
gacaccatca acgaggaggc cggcgagttt gacccgtgc accccgtgca cggccggcc 660  
atcgcccccg gccagatgcg cgagcccccgc ggcagcgcaca tcgcggcgcac caccagcacc 720  
ctgcaggagc agatgcctg gatgaccagc aaccccccaca tccccgtggg cgacatctac 780

aagcgggtgga tcatcctggg cctgaacaag atcgtgcgga tgtacagccc cgtgagcatc 840  
 ctggacatca agcaggggccc caaggagcc ttccgcact acgtggaccg cttttaag 900  
 accctgcgcg ccgagcagag cacccaggag gtgaagaact ggatgaccga caccctgctg 960  
 gtgcagaacg ccaaccccgaa ctgcaagacc atcctgcgcg ctctcgcccc cggccgcagc 1020  
 ctggaggaga ttagtgcgcg ctgcgcggc gtggggccgc ccagccacaa ggcccgcgtg 1080  
 ctggccgagg cgatgagcca ggcacaccc agcgtatga tgcaagaagag caacttcaag 1140  
 ggcgcgcgcg gcacatgtcaa gtgcgttcaac tgccgcagg agggccacat cgccgcac 1200  
 tgccgcgcgc cccgcacaa gggctgtgg aagtgcggca aggagggcca ccagatgaag 1260  
 gactgcaccg agcgcgcaggc caacttcctg ggcaagatct ggcccagcca caagggccgc 1320  
 cccggcaact tcctgcagag ccgcggccgc cccaccgcgc ccccccgcga gagcttccgc 1380  
 ttcgaggaga ccaccccgaa ccagaagcag gagagcaagg accgcgagac cctgaccagc 1440  
 ctgaagagcc tggcggcaa cgacccctg agccagtaa 1479

<210> 4  
 <211> 1509

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Gag  
 of HIV strain AF110967

<400> 4

atggggccgc ggcgcgcgc cctgcgcggc gagaagctgg acaagtggga gaagatccgc 60  
 ctgcgcgcgc gggcaagaa gcaactacatg ctgaagcacc tgggtgtggc cagccgcgag 120  
 ctggagggtc tcgcctgaa ccccgccctg ctggagaccg ccgaggggctg caagcagatc 180  
 atgaagcgcg tgcagccgc cctgcagacc ggcacccgagg agctgcgcgc cctgtacaac 240  
 accgtggcca ccctgtactg cgtgcacgc ggcacatcgagg tccgcgcacac caaggaggcc 300  
 ctggacaaga tcgaggaggaa gcagaacaag tcccgacaga agaccccgca ggccaaggag 360  
 gcccacggca aggtgagcca gaactacccc atcgtgcaga acctgcagg ccagatgg 420  
 caccaggcca tcagcccccg caccctgaac gcctgggtga aggtgatcg ggagaaggcc 480  
 ttcagcccccg aggtgatccc catgttccacc gccttgacgc agggcgccac ccccccaggac 540  
 ctgaacacga tggtaaacac cgtggccgc caccaggccg ccatgcacat gctgaaggac 600  
 accatcaacgc aggaggccgc cgagtggac cgcctgcacc ccgtgcaggc cggcccccgt 660  
 gccccccggcc agatgcgcga ccccccggc agcgcacateg cccggccac cagcacccctg 720  
 caggagcaga tcgcctggat gaccagcaac ccccccgtgc ccgtggggcga catctacaag 780  
 cggtggatca tcctgggcct gaacaagatc gtgcggatgt acagccccgt gaggatcctg 840  
 gacatccgcc agggcccaa ggagccctc cgcgactacg tggaccgctt cttcaagacc 900  
 ctgcgcgcgc agcaggccac ccaggacgtg aagaactggg tgaccgagac cctgctgg 960  
 cagaacgcca acccccgactg caagacccatc ctgcgcgcgc tcggcccccgg cggccacccctg 1020  
 gaggagatga tgaccgcctg ccaggcggtg ggcggccccc gccacaaggc cgcgtgtctg 1080  
 gccgaggcga tgagccaggc caacagcggtg aacatcatga tgcaagaagag caacttcaag 1140  
 ggcgcgcgcg gcaacgtcaa gtgcgttcaac tgccgcagg agggccacat cgccaaagAAC 1200  
 tggcgcgcgc cccgcacaa gggctgtgg aagtgcggca aggagggcca ccagatgaag 1260  
 gactgcaccg agcgcgcaggc caacttcctg ggcaagatct ggcccagcca caagggccgc 1320  
 cccggcaact tcctgcacaa ccgcacggc cccggccccc ccaccgtgc caccggccccc 1380  
 cccggccaga gctcccgctt cgaggagacc acccccgccc ccaaggcaggaa gcccaaggac 1440  
 cgcgaggccct accgcgagcc cctgaccgc ctgcgcagcc tggcggcag cggcccccgt 1500  
 agccagtaa 1509

<210> 5

<211> 141

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Env common  
 region of HIV strain AF110968

<400> 5  
 accatcacca tcacctgccg catcaagcag atcatcaaca tgtggcagaa ggtggccgc 60  
 gccatgtacg ccccccccat cgccggcaac ctgacctgcg agagcaacat caccggctg 120  
 ctgctgaccc gcgacggcgg c 141

<210> 6  
 <211> 1431  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 gp120 coding region of HIV strain AF110968

<400> 6  
 agcgtgggtgg gcaacctgtg ggtgaccgtg tactacggcg tgcccgtgtg gaaggaggcc 60  
 aagaccaccc ttttctgcac cagcgacgcc aaggcctacg agaccgaggt gcacaacgtg 120  
 tggccaccc acgcctgcgt gcccacccg cccaaacccccc aggagatcgt gctggagaac 180  
 gtgaccgaga acttcaacat gtggaaagac gacatggtgg accagatcga cgaggacatc 240  
 atcagcctgt gggaccagag cctgaagccc tgcgtgaagc tgaccccccgt gtgcgtgacc 300  
 ctgaagtgcc gcaacgtgaa cgccaccaac aacatcaaca gcatgatcga caacagcaac 360  
 aaggccgaga tgaagaactg cagcttcaac gtgaccaccc agctgcgcga ccgcaagcag 420  
 gaggtgcacg ccctgttcta ccgcctggac gtggcggcc tgcaggccaa caacagcaac 480  
 gagtaccgcg tcatcaactg caacaccagg gccatcaccc aggcctggcc caaggtgagc 540  
 ttgcacccca tccccatcca ctactgcacc cccggccgtt acgcateet gaagtgcac 600  
 aaccagaccc tcaacggcac cggccctgc aacaacgtga gcatggtgca gtgcgcac 660  
 ggcatcaagc ccgtggtgag caccacgtg ctgcgtgaacg gcatgcggc caagggcgag 720  
 atcatcatcc gcaagcgagaa cctggccaaac aacgccaaga tcatcatcgt gcatgtgac 780  
 aagccctgtg agatcgtgtg cgtgcgcggc aacaacaaca cccgcagaa cgtgcgcatac 840  
 ggccccggcc agacccctta cgccacccggc gagatcatcg gcatgcaccc ccaggccatc 900  
 tgcattatca acaagaccga gtggaaacagc accctgcagg gcatgcggcaaa gaagctggag 960  
 gagcaattca gcaagaaggc catcaagttc gagcccgac gcccggccg cctggagatc 1020  
 accaccacca gttcaactg ccgcggccgg ttccttact gcatgcaccc ccagctgttc 1080  
 aacagcaccc acagcccccag cttcaacccggc accgagaaca agctgaacgg caccatcacc 1140  
 atcacttgc gcatcaagca gatcatcaac atgtggcaga aggtggccgt ccgcattgtac 1200  
 gccccccca tcgccccggca cctgacccgtc gagagcaaca tcaaccggct gcatgtgacc 1260  
 cgcacccggc gcaagaccgg ccccaacccac accgagatct tccggccggcc cggccggccgac 1320  
 atgcgcgaca actggcgcac ctagctgtac aagtacaagg tgggtggagat caagccctg 1380  
 ggcgtggccc ccaccggaggc caagcgccgc gtggtggagc gcgagaagcg c 1431

<210> 7  
 <211> 1944  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 gp140 coding region of HIV strain AF110968

<400> 7  
 agcgtgggtgg gcaacctgtg ggtgaccgtg tactacggcg tgcccgtgtg gaaggaggcc 60  
 aagaccaccc ttttctgcac cagcgacgcc aaggcctacg agaccgaggt gcacaacgtg 120  
 tggccaccc acgcctgcgt gcccacccg cccaaacccccc aggagatcgt gctggagaac 180  
 gtgaccgaga acttcaacat gtggaaagac gacatggtgg accagatcga cgaggacatc 240  
 atcagcctgt gggaccagag cctgaagccc tgcgtgaagc tgaccccccgt gtgcgtgacc 300  
 ctgaagtgcc gcaacgtgaa cgccaccaac aacatcaaca gcatgatcga caacagcaac 360  
 aaggccgaga tgaagaactg cagcttcaac gtgaccaccc agctgcgcga ccgcaagcag 420  
 gaggtgcacg ccctgttcta ccgcctggac gtggcggcc tgcaggccaa caacagcaac 480  
 gagtaccgcg tcatcaactg caacaccagg gccatcaccc aggcctggcc caaggtgagc 540

ttcgacccca tccccatcca ctactgcacc cccggcgct acgccatcct gaagtgcac 600  
 aaccagacct tcaacggcac cgccccctgc aacaacgtga gcagcgtgca gtgcgcccac 660  
 ggcataaggc ccgtggtag cccccagct ctgtgaacg gcagcctggc caaggcgag 720  
 atcatcatcc gcaagcgagaa cctggcaac aacgccaaga tcatcatcg gcagctgaac 780  
 aagcccgta agatcggtg cgtgegccc aacaacaaca cccgcaagag cgtgcgcate 840  
 ggccccggcc agaccttcta cgccaccggc gagatcatcg ggcacatccg ccaggcctac 900  
 tgcatcatca acaagaccga gtggAACAGC accctgcagg gcgtgagcaa gaagctggag 960  
 gagcacttca gcaagaaggc catcaaggc gagcccagca gggggggcga cttggagatc 1020  
 accacccaca gttcaactg cccggcgag ttcttact ggcacaccag ccagctgttc 1080  
 aacagcacct acagccccag cttcaacggc accgagaaca agctgaacgg caccatcacc 1140  
 atcacttgc gcatcaagca gatcatcaac atgtggcaga aggtggccg cgcctatgtac 1200  
 gccccccca tcgccccaa cctgacctgc gagagcaaca tcacccggct gctgtgacc 1260  
 cgcacggcg gcaagaccgg ccccaacgac accgagatct tccggggggg cggcgccgac 1320  
 atgcgcgaca actggcgcaa cgagctgtac aagtacaagg ttgtggagat caagccctg 1380  
 ggcgtggccc ccaccggagc caagcgccgc tggtggagc gcgagaagcg cggcgccggc 1440  
 atcggcgccg tttcttggg cttcttggc gcccggca gcaccatggg cggcgccgac 1500  
 atcaccctga ccgtgcaggc cccctgtc ctgagcggca tcgtgcagca gcagaacaac 1560  
 ctgctgcgcg ccacatcgaggc ccagcagcac ctgctgcagc tgaccgtgtg gggcatcaag 1620  
 cagctgcaga cccgcacccctt ggccgtggag cgctacatcg aggaccagca gctgtgggc 1680  
 atctggggct gcaacggcaa gctgatctgc accaccggc tgccctggaa cagcagctgg 1740  
 agcaaccgca gcaacggacca gatctggac aacatgaccc gatgcagtg ggaccggag 1800  
 atcaacaact acaccgacac catctaccgc ctgctggagg agagccagaa ccagcaggag 1860  
 aagaacgaga agacactgtt ggcctggac agctggcaga acctgtggaa ctggttcagc 1920  
 atcaccactt ggctgtggta catc 1944

&lt;210&gt; 8

&lt;211&gt; 2466

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
gp160 coding region of HIV strain AF110968

&lt;400&gt; 8

agcgtggtag gcaacctgtg ggtgaccgtg tactacggcg tgccctgtg gaaggaggcc 60  
 aagaccaccc tttctgcac cagcgaccc aaggctacg agaccgaggt gcacaacgtg 120  
 tggccaccc accgcctgcgt gcccaccggc cccaaaaaccaggatcgatcgatcg 180  
 gtgacccgaga acttcaacat gtggaaac gacatggtag accagatcgatcgatcg 240  
 atcagctgtt gggaccaggag cctgaagccc tgcgtgaagc tgaccccccgttgcgtgacc 300  
 ctgaagtgc gcaacgtgaa cggccaccaac aacatcaaca gcatgtatcgatcgatcg 360  
 aaggccgaga tgaagaactg cagcttcaac gtgaccaccgg agctgcgcgatcgatcg 420  
 gaggtgcacg cccctttctt cccctgtggac tggtggccccc tgcaggggcaaaacagcaac 480  
 gagtaccgc tcatcaactg caacaccggc gccatcaccc accgcctgccc caaggtgagc 540  
 ttcgacccca tccccatcca ctactgcacc cccggcgct acgccatcctt gaagtgcac 600  
 aaccagacct tcaacggcac cgccccctgc aacaacgtga gcagcgtgca gtgcgcccac 660  
 ggcataaggc ccgtggtag caccctgtc ctgatcgatcgatcgatcgatcgatcg 720  
 atcatcatcc gcaagcgagaa cttggccacca aacgccaaga tcatcatcgatcgatcg 780  
 aagcccgta agatcggtg cgtgcgcggc aacaacaaca cccgcaagag cgtgcgcate 840  
 ggccccggcc agaccttcta cgccaccggc gagatcatcg ggcacatccg ccaggcctac 900  
 tgcatcatca acaagaccga gtggaaacgac accctgcagg gcgtgagcaa gaagctggag 960  
 gagcacttca gcaagaaggc catcaaggc gagcccagca gggggggcga cttggagatc 1020  
 accacccaca gttcaactg cccggcgag ttcttact ggcacaccag ccagctgttc 1080  
 aacagcacct acagccccag cttcaacggc accgagaaca agctgaacgg caccatcacc 1140  
 atcaccctgca gcatcaagca gatcatcaac atgtggcaga aggtggccg cgcctatgtac 1200  
 gccccccca tcgccccaa cctgacctgc gagagcaaca tcacccggct gctgtgacc 1260  
 cgcacggcg gcaagaccgg ccccaacgac accgagatct tccggggggg cggcgccgac 1320  
 atgcgcgaca actggcgcaa cgagctgtac aagtacaagg ttgtggagat caagccctg 1380  
 ggcgtggccc ccaccggagc caagcgccgc gtggggagc gcgagaagcg cggcgccggc 1440

atcggccgc tgttcctggg cttcctggc gcccggca gcaccatggg cgccgccagc 1500  
 atcacctga ccgtgcaggc ccgcctgcg ctgagcggca tcgtgcagca gcagaacaac 1560  
 ctgctgcgcg ccatcgaggc ccagcagcac ctgctgcagc tgacctgtg gggcatcaag 1620  
 cagctgcaga cccgcattct ggccgtggag cgctacctga aggaccagca gctgctggc 1680  
 atctggggct gcagcggcaa gctgatctgc accacccggc tgccctggaa cagcagctgg 1740  
 agcaaccgca gccacgcgaa gatctggac aacatgacct ggtgcagtg ggaccgcgag 1800  
 atcaacaact acaccgacac catctaccgc ctgctggagg agagccagaa ccagcaggag 1860  
 aagaacgaga aggacctgtt ggcctggac agctggcaga acctgtggaa ctggttcagc 1920  
 atcaccaact ggctgtggta catcaagatc ttcatcatga tcgtggggc cctgatccgc 1980  
 ctgcgcatac tcttcgcgt gctgagcatac gtgaacccggc tgccgcaggg ctacagcccc 2040  
 ctgccttcc agaccctgac ccccaacccc cgcgagcccg accgcctggg ccgcattcag 2100  
 gaggaggcgc gcgagcagga cgcggccgc agcatccgc tggtagcgg cttectggcc 2160  
 ctggcttggg acgacctgcg cagcctgtgc ctgttcaact accaccgcct ggcgcacttc 2220  
 atcctgatcg ccccccgcgt gctggagctg ctggccagc gcccgtggaa ggccctgaag 2280  
 tacctggca gcctgggtca gtactgggc ctggagctga agaagagcgc catcagcctg 2340  
 ctggacacca tcgcacatgcg cgtggccgag ggcacccgacc gcatcatcga gttcatccag 2400  
 cgcacatgcg cgcacatccg caacatcccc cgccgcatacc gccagggtt cgaggccgc 2460  
 ctgcag 2466

<210> 9  
 <211> 2547  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 signal sequence and gp160 coding region of HIV  
 strain AF110968

<400> 9

atgcgcgtga tggcattct gaagaactac cagcagtggt ggatgtgggg catcctggc 60  
 ttctggatgc tgatcatcg cagcgtggg ggcaacctgt ggtgaccgt gtactacggc 120  
 gtgcccgtgt ggaaggaggc caagaccacc ctgttctgca ccagcgcacgc caaggcctac 180  
 gagaccgggg tgacacaacgt gtggggccacc cacgcctgcg tgccctaccga ccccaacccc 240  
 caggagatcg tgctggagaa cgtgaccggag aacttcaaca tggttggaaa cgacatgtg 300  
 gaccatcgatcg acggaggacat catcagccgt tgggaccaga gcctgaagcc ctgcgtgaag 360  
 ctgacccccc tggcggtac cctgaagtgc cgcaacgtga acggccacca caacatcaac 420  
 agcatatcg acaacagcaa caagggcggag atgaagaact gcaatgttca cgtgaccacc 480  
 gagctgcgcg acggcagaa ggagggtgcac gcccgtttt actgccttgg cgtgggtccc 540  
 ctgcaggggca acaacagcaa cgatgttccg ctgatcaact gcaacaccag cgccatcacc 600  
 caggcctgccc ccaaggttag ctgcacccatcc actactgcac ccccgccggc 660  
 tacgcacatcc tgaagtgcac caaccagacc ttcaacggca cccggccctg caacaacgtg 720  
 agcagcgtgc agtgcgttcc caaggcatcaag cccgtggta gcaacccagct gctgctgaac 780  
 ggcagcctgg ccaagggcga gatcatcatc cgccggaga acctggccaa caacgccaag 840  
 atcatcatcg tgcagctgaa caagcccgtg aagatcggt gctgtgcggcc caacaacaac 900  
 acccgcaaga gctgcgcacatcc cggcccccggc cagacccatcc acggccacccgg cgagatcatc 960  
 ggcgcacatcc gccaggccta ctgcacatcatc aacaagaccg agtggaaacag caccctgcac 1020  
 ggcgtgagca agaagctgga ggagcacttc agcaagaagg ccatcaagtt cgagcccaag 1080  
 agcggccggcg acctggagat caccaccac agcttcaact gccgcggcga gtttttctac 1140  
 tgcgcacacca gccagctgtt caacagcacc tacagccca gttcaacgg caccgagaac 1200  
 aagctgaacg gcaaccatcac catcacatgcg cgccatcaagc agatcatcaa catgtggcag 1260  
 aagggtggcc gcccgcattgtt cgcggccatcc atgcgcggca acctgcacccgt cgagagcaac 1320  
 atcacccggcc tgcgtgcac ccgcgcacggc ggcaagaccg gccccaacga caccgcgatc 1380  
 ttccgcggcc gcccgcggcga catgcgcgac aactggcga accagctgtt caagtacaag 1440  
 gtgggtggaga tcaaggccctt gggcggtggc cccacccggg ccaagcgcggc cgtgggtggag 1500  
 cgccgagaagg gcccgcgtggc catcgccgcgtt gtgttccctgg gttccctggg cgcgcggcc 1560  
 agcaccatgg gcccgcggcag catcacccatcc accgtgcagg cccgcctgtt gctgagcggc 1620  
 atcgtgcagc agcagaacaa cctgcgtgcgc gccatcgagg cccagcagca cctgcgtcag 1680  
 ctgaccgtgtt gggcatcaa gcaatgcag accccgcatacc tggccgtggaa ggcgttccctg 1740

aaggaccagc agctgctggg catctggggc tgcagcggca agctgatctg caccacccgc 1800  
 gtgccttgc acacgagctg gagcaaccgc agccacgacg agatctggga caacatgacc 1860  
 tggatgcagt gggaccgcga gatcaacaac tacaccgaca ccatctaccg cctgtggag 1920  
 gagagccaga accagcagga gaagaacgag aaggacctgc tgccctggc cagctggcag 1980  
 aacctgtgg aactggttcag catcaccaac tggctgtggt acatcaagat cttcatcatg 2040  
 atcgtggcg gcctgatcgg cctgcgcattc atcttcgcgg tgctgagcat cgtgaaccgc 2100  
 gtgcgcagg gctacagccc cctgcgcctc cagaccccta ccccccaaccc ccgcgagccc 2160  
 gaccgcctgg gcccgcata gggaggaggc ggcgagcagg accgcggccg cagcatccgc 2220  
 ctggtgagcg gcttcctggc cctggctgg gacgacctgc gcaagctgtg cctgttcage 2280  
 taccacccgc tgcgcgactt catcctgate gccgcggcg tgctggagct gctggccag 2340  
 cgccggctggg aggccctgaa gtacctggc agcctgtgc agtactgggg cctggagctg 2400  
 aagaagagcg ccatcagect gctggacacc atcgcattcg ccgtggccga gggcaccgcac 2460  
 cgcacatcg agttcatcca ggcacatcg cgcgcattcc gcaacatccc ccgcggcattc 2520  
 cgccaggcgct tcgaggccgc cctgcag 2547

<210> 10  
<211> 1035  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic a  
gp41 coding region of HIV strain AF110968

<400> 10  
gccgtggca tcggcgccgt gttcctgggc ttccctggcg ccgcggcag caccatggc 60  
gccgcagca tcacccctgac cgtgcaggcc cgcctgcgc tgagcggcat cgtgcagcag 120  
cagaacaacc tgctgcgcgc catcgaggcc cagcagcacc tgctgcagct gaccgtgtgg 180  
ggcatcaagc agctgcagac ccgcattctg ggcgtggage gtcacctgaa ggaccagcag 240  
ctgctggca tctgggctg cagcggcaag ctgatctgca ccacccgcgt gcccctggaaac 300  
agcagctgga gcaaccgcag ccacgcgcag atctggaca acatgacctg gatgcagttg 360  
gaccgcgaga tcaacaacta caccgcaccc atctaccgc tgctggagga gagccagaac 420  
cagcaggaga agaacgcgaaa ggacctgtg gcccctggaca gtcggcagaa cctgtggaaac 480  
tggttcagca tcaccaactg gctgtggtaatc acatcaagatct tcatcatgtat cgtggccggc 540  
ctgatcgccc tgcgcatcat cttcgccgtg ctgagcatcg tgaaccgcgt ggcgcaggc 600  
tacagcccccc tgcccttcca gaccctgacc cccaaaaaaa gcgagccgcg cccgcctggc 660  
cgcatcgagg aggaggccgg cgagcaggac cgcggccgcgac gcatccgcct ggtgagccgc 720  
ttccctggccc tggctggca cgcattgcgc agcctgtgc tggtcagcta ccacccgcctg 780  
cgacttcac tcctgatgc cgcggcggtg ctggagctgc tgccgcggcg cggctggag 840  
gcccctgaagt acctggcag cctgggtgcag tactggggc tggagctgaa gaagagcgcac 900  
atcagcctgc tggacaccat cgcattcgcc gtcggccgagg gcaaccgcaccc catcatcgag 960  
ttcatccacgc gcatctgccc cgcattccgc aacatcccccc gccgcattccg ccaggcccttc 1020  
gaggccgcccc tgca 1035

<210> 11  
<211> 144  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: synthetic Env  
common region of HIV strain AF110975

<400> 11  
agcatcatca ccctgcctg ccgcattcaag cagatcatcg acatgtggca gaaggtggc 60  
cgccatct acggccccc catcgaggcc aacatcacct gcagcagcag catcaccggc 120  
ctgctgtgg cccgcgacgg cgcc 144

<210> 12

&lt;211&gt; 1437

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
gp120 coding region of HIV strain AF110975

&lt;400&gt; 12

agcggcctgg gcaacctgtg ggtgaccgtg tacgacggcg tgcccgtgt gcgcgaggcc 60  
 agcaccaccc ttttctgcgc cagcgacgcc aaggcctacg agaaggaggt gcacaacgtg 120  
 tggggccaccc acgcctgcgt gcccacccgac cccaaaccccc aggagatcga gctggacaac 180  
 gtgaccgaga acttcaacat gtggagaac gacatggtgg accagatgca cgaggacatc 240  
 atcagcctgt gggaccagag cctgaagccc cgcgtgaagc tgaccccccct gtgcgtgacc 300  
 ctgaagtgc ccaactacag caccaactac agcaacacca tgaacgcccac cagctacaac 360  
 aacaacacca ccgaggagat caagaactgc accttcacca tgaccacccga gctgcgcgac 420  
 aagaagcgc aggtgtacgc cctgttctac aagctggaca tcgtgcccct gaacagcaac 480  
 agcagcggat accgcctgtat caactgcaac accagcgcca tcacccaggc ctgccccaaag 540  
 gtgagttcg accccatccc catccactac tgccggcccg cccgctacgc catcctgaag 600  
 tgcaagaaca acaccagcaa cggcacccgc ccctggcaga acgtgagcac cgtcagatgc 660  
 acccacccgc tcaagccgt ggtgagcacc cccctgctgc tgaacggcag cctggccgag 720  
 ggcggcgaga tcatcatccg cagcaagaac ctgagcaaca acgcctacac catcatcg 780  
 cacctgaacg acagcgtgga gatcgtgtgc acccgccccca acaacaacac cccgcaaggcc 840  
 atccgcacatcg gccccggcca gaccttctac gccaccgaga acatcatcg cgacatccgc 900  
 caggccccact gcaacatcag cggccggcag tggacaacagg ccgtgcagcg cgtgagcgc 960  
 aagctgcgcg agcacttccc caacaagacc atcaggttcc agcccaagcag cggccggcgc 1020  
 ctggagatca ccacccacag cttcaactgc cgcggcgagt ttttctactg caacaccaggc 1080  
 aagctgttca acagcagcta caacggcacc agtaccgcg gcaccggagag caacaggcgc 1140  
 atcatcaccc tgccctgcgc catcaaggag atcatcgaca tgtggcagaa ggtggccgc 1200  
 gccatctacg ccccccacat cgagggcaac atcacctgca gcagcagcat caccggcctg 1260  
 ctgctggccc ggcacccggg cctggacaac atcaccaccc agatcttccg ccccccaggcc 1320  
 ggcgacatga aggacaactg ggcacccggg ctgtacaagt acaagggtgt ggagatcaag 1380  
 cccctggccg tggcccccac cgaggccaag cgccgcgtgg tggagcgcga gaagcgc 1437

&lt;210&gt; 13

&lt;211&gt; 1950

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
gp140 coding region of HIV strain AF110975

&lt;400&gt; 13

agcggcctgg gcaacctgtg ggtgaccgtg tacgacggcg tgcccgtgt gcgcgaggcc 60  
 agcaccaccc ttttctgcgc cagcgacgcc aaggcctacg agaaggaggt gcacaacgtg 120  
 tggggccaccc acgcctgcgt gcccacccgac cccaaaccccc aggagatcga gctggacaac 180  
 gtgaccgaga acttcaacat gtggagaac gacatggtgg accagatgca cgaggacatc 240  
 atcagcctgt gggaccagag cctgaagccc cgcgtgaagc tgaccccccct gtgcgtgacc 300  
 ctgaagtgc ccaactacag caccaactac agcaacacca tgaacgcccac cagctacaac 360  
 aacaacacca ccgaggagat caagaactgc accttcacca tgaccacccga gctgcgcgac 420  
 aagaagcgc aggtgtacgc cctgttctac aagctggaca tcgtgcccct gaacagcaac 480  
 agcagcggat accgcctgtat caactgcaac accagcgcca tcacccaggc ctgccccaaag 540  
 gtgagttcg accccatccc catccactac tgccggcccg cccgctacgc catcctgaag 600  
 tgcaagaaca acaccagcaa cggcacccgc ccctggcaga acgtgagcac cgtcagatgc 660  
 acccacccgc tcaagccgt ggtgagcacc cccctgctgc tgaacggcag cctggccgag 720  
 ggcggcgaga tcatcatccg cagcaagaac ctgagcaaca acgcctacac catcatcg 780  
 cacctgaacg acagcgtgga gatcgtgtgc acccgccccca acaacaacac cccgcaaggcc 840  
 atccgcacatcg gccccggcca gaccttctac gccaccgaga acatcatcg cgacatccgc 900

caggccccact gcaacatcag cgccggcgag tggaaacaagg ccgtgcagcg cgtgagcgcc 960  
 aagctgcgcg agcacttccc caacaagacc atcgagttcc agcccgagcg cggcgccgac 1020  
 ctggagatca ccacccacag cttcaactgc cgccgcgagt ttttctactg caacaccagc 1080  
 aagctgttca acacgcgcta caacggcacc agtaccgcg gcaccgagag caacagcagc 1140  
 atcatcaccc tgccctgcgg catcaagcag atcatcgaca tgtggcagaa ggtggccgc 1200  
 gccatctacg cccccccat cgagggcaac atcacctgca gcagcagcat caccggcctg 1260  
 ctgctggccc ggcacggcg cctggacaac atcaccaccc agatttccg ccccccaggc 1320  
 ggcacatga aggacaactg ggcacacgag ctgtacaagt acaagggttgt ggagatcaag 1380  
 cccctggcg tggccccac cgaggccaag cgccgcgtgg tggagcgcga gaagcgcgcc 1440  
 gtgggcattcg ggcgcgtgat cttcggttc ctggcgccg cccgcagcaa catggcgcc 1500  
 gccagcatca ccctgaccgc ccaggcccgc cagctgctga gcggcatcg gcagcagcag 1560  
 agcaacctgc tgcgcccat cgaggcccag cagcacatgc tgcaagctgac cgtgtgggc 1620  
 atcaagcagc tgcaaggccc cgtgtggcc atcgagcgct acctgaagga ccagcagctg 1680  
 ctgggcattcg gggcgtcag cggcaagctg atctgcacca ccaccgtgcc ctggAACAGC 1740  
 agctggagca acaagaccca gggcgagatc tggagaaca tgacctggat gcagtggac 1800  
 aaggagatc gcaactacac cggcatcatac taccgcctgc tggaggagag ccagaaccag 1860  
 caggcaga acgagaagga cctgtggcc ctggacagcc gcaacaacct gtggagctgg 1920  
 ttcaacatca gcaactggct gtggtacatc 1950

<210> 14  
 <211> 2493  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic  
 gp160 coding region of HIV strain AF110975

<400> 14  
 agcggcctgg gcaacctgtg ggtgaccgtg tacgacggcg tgcccggttg ggcgcaggcc 60  
 agcaccaccc tggctcgcc cagcgacgcc aaggcctacg agaaggaggt gcacaacgtg 120  
 tggccaccc acgcctgcgt gcccacccgc cccaaacccccc aggagatcga gctggacaac 180  
 gtgacccgaga acttcaacat gtggaaaac gacatggtgg accagatgcg cgaggacatc 240  
 atcagcctgt gggaccagag cctgaagccc cgcgtgaagc tgaccccccgt gtgcgtgacc 300  
 ctgaagtgc ccaactacag cacaactac agcaacacca tgaacgcac cagtcataaac 360  
 aacaacacca cggaggatg caagaactgc accttcaaca tgaccacccga gctgcgcgc 420  
 aagaaggcgc aggtgtacgc cctgttctac aagctggaca tcgtgccccctt gaacagcaac 480  
 agcagcgcgt accgcctgtat caactgcac accagcgcac tcacccaggc ctgccccaa 540  
 gtgagcttcg accccatccc catccactac tgccgcggcc cccgcgtacgc catctgaag 600  
 tgcaagaaca acaccagcaa cggcacccggc ccctgccaga acgtgagcac cgtgcagtgc 660  
 accccacggca tcaagcccggt ggtgagcaccc cccctgctgc tgaacggcag cctggccgag 720  
 ggcggcgaga tcatcatccg cagcaagaac ctgagcaaca acgcctacac catcatcg 780  
 cacctgaacg acagcgtgga gatcgtgtgc accccgcacca acaacaacac cggcaaggc 840  
 atccgcacatcg gccccggcca gacccatcg gccacccgaga acatcatcg cgacatccgc 900  
 caggcccaact gcaacatcag cgcggcgag tggaaaagg ccgtgcagcg cgtgagcgcc 960  
 aagctgcgcg agcacttccc caacaagacc atcgagttcc agcccgagcg cggcgccgac 1020  
 ctggagatca ccacccacag cttcaactgc cgccgcgttgt ttttctactg caacaccagc 1080  
 aagctgttca acacgcgcta caacggcacc agtaccgcg gcaccgagag caacagcagc 1140  
 atcatcaccc tgccctgcgg catcaagcag atcatcgaca tgtggcagaa ggtggccgc 1200  
 gccatctacg cccccccat cgagggcaac atcacctgca gcagcagcat caccggcctg 1260  
 ctgctggccc ggcacggcg cctggacaac atcaccaccc agatttccg ccccccaggc 1320  
 ggcacatga aggacaactg ggcacacgag ctgtacaagt acaagggttgt ggagatcaag 1380  
 cccctggcg tggccccac cgaggccaag cgccgcgtgg tggagcgcga gaagcgcgcc 1440  
 gtgggcattcg ggcgcgtgat cttcggttc ctggcgccg cccgcagcaa catggcgcc 1500  
 gccagcatca ccctgaccgc ccaggcccgc cagctgctga gcggcatcg gcagcagcag 1560  
 agcaacctgc tgcgcccat cgaggcccag cagcacatgc tgcagctgac cgtgtgggc 1620  
 atcaagcagc tgcaaggccc cgtgtggcc atcgagcgct acctgaagga ccagcagctg 1680  
 ctgggcattcg gggcgtcag cggcaagctg atctgcacca ccaccgtgcc ctggAACAGC 1740  
 agctggagca acaagaccca gggcgagatc tggagaaca tgacctggat gcagtggac 1800

aaggagatca gcaactacac cggcatcatc taccgcctgc tggaggagag ccagaaccag 1860  
 caggagcaga acgagaagga cctgctggcc ctggacagcc gcaacaacct gtggagctgg 1920  
 ttcaacatca gcaactggct gtggtacatc aagatcttca tcatgatcg gggcggctg 1980  
 atcggcctgc gcatcatctt cgccgtgctg agcatcgta accgcgtcg ccaggctac 2040  
 agccccctga gttccagac cctgacccccc aaccccccgcg gcctggaccg cctggccgc 2100  
 atcgaggagg agggcggcga gcaggaccgc gaccgcagca tccgcctggt gcaggcttc 2160  
 ctggccctgg cctggacga cctgcgcagc ctgtgcctgt tcagctacca cgcctgcgc 2220  
 gacctgatec ttgtgaccgc cccgcgtggt gagctgtgg gcccgcagcg ccccccgcgc 2280  
 ctgcagcgcg gctgggaggc cctgaagtac ctgggcagcc ttgtgcagta ctgggctg 2340  
 gagctgaaga agagcgcac cagcctgtg gacagcatcg ccategcgcgt ggccgagggc 2400  
 accgaccgca tcatcgaggat gatccagcgc atctaccgcg cttctgcaa catccccgc 2460  
 cgcgtgcgcc agggcttcga ggccgcctg cag 2493

&lt;210&gt; 15

&lt;211&gt; 2565

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: synthetic  
signal sequence and gp160 coding region of HIV  
strain AF110975

&lt;400&gt; 15

atgcgcgtgc gcggcattct gcgcagctgg cagcagtggt ggatctgggg catcctggc 60  
 ttctggatct gcagcggcct gggcaacctg tgggtgaccg ttagtgcacgg cgtgcccgtg 120  
 tggcgcgagg ccagcaccac cctgttctgc gccagcgcacg ccaaggccctaa cgagaaggag 180  
 gtgcacaacg tggggccac ccacgcctgc gtgcgcaccc accccaaccc ccaggagatc 240  
 gagctggaca acgtgaccga gaacttcaac atgtggaaga acgacatggt ggaccagatg 300  
 cacgaggaca tcatcagccgt gtgggaccag agcctgaagc cccgcgtgaa gtcgacccccc 360  
 ctgtgcgtga ccctgaagtg caccacatc agcacaact acagcaacac catgaacgc 420  
 accagctaca acaacaacac caccgaggag atcaagaact gcaccttcaa catgaccacc 480  
 gagctgcgcg acaagaagca gcaggtgtac gcccgtttt acaagctgga catcggtccc 540  
 ctgaacagca acagcgcga gtaccgcctg atcaactgca acaccagcgc catcacccag 600  
 gcctgcctca aggtgagtt cgacccatc cccatccact actgcgecccc cgccggctac 660  
 gccatctga agtgcagaa caacaccagg aacggcaccg gcccgtgca gaacgtgagc 720  
 accgtgcgt gcaaccacgg catcaagccc gtggtgagca ccccccgtgt gctgaacggc 780  
 agcctgcccgg aggccggcga gatcatcata cgcagcaga acctgagcaa caacgcctac 840  
 accatcatcg tgcacctgaa cgacagcgtg gagatcggtt gcacccggcc caacaacaac 900  
 acccgcaagg gcatccgcat cggcccccgc cagacattt acgcacccga gaacatcatc 960  
 ggcgacatcc gccaggccca ctgcaacatc agcgcggcg agtggaaacaa ggcctgcag 1020  
 cgcgtgagcg ccaagctgcg cgagcacttc cccacaaga ccatcgagtt ccagcccgac 1080  
 agcggccggcg acctggagat caccacccac agcttcaact gcccggcga gtttttac 1140  
 tgcaacacca gcaagctgtt caacagcgc tacaacggc ccaagctaccg cggcaccggag 1200  
 agcaacagca gcatcatcac cctgccttcgc cgcataaggc agatcatcg catgtggcag 1260  
 aaggtggcc gcccacatcta cggcccccgg atcgagggca acatcacctg cagcagcagc 1320  
 atcaccggcc tgctgctggc cgcgcacggc ggcctggaca acatcaccac cgagatctt 1380  
 cggcccccagg gcggcgcacat gaaggacaac tggcgcacg agctgtacaa gtacaagggt 1440  
 gtggagatca agcccttggg cgtggccccc accgaggcca aegccgcgt ggtggagcgc 1500  
 gagaaggcgc cctggggcat cggcgcgcgtg atcttcggct tccctggcgc cggccggcagc 1560  
 aacatgggcg cccgcacat caccctgacc gcccaggccc gccagctgt gacggcatac 1620  
 gtgcagcgc agagcaaccc gctgcgcggc atcgaggccc agcagcacaat gctgcagctg 1680  
 accgtgtggg gcatcaagca gctgcaggcc cgcgtgtgg ccatcgagcg ctacatcgaa 1740  
 gaccagcgc tgcgtggcat ctggggctgc agcggcacaagc tgatctgcac caccaccgt 1800  
 ccctggaaaca gcaagctggag caacaagacc cagggcgcaga tctgggagaa catgacccgt 1860  
 atcgatgggg acaaggagat cagcaactac accggcatac ttcaccgcct gctggaggag 1920  
 agccagaacc agcaggagca gaacgagaag gacctgtgg ccctggacag cgcacaaac 1980  
 ctgtggagct ggtcaacat cagcaactgg ctgtgttaca tcaagatctt catcatgatc 2040  
 gtggccggcc tgcgtgcgc tgcgtgcgc tgacatcgatc gacccgcgtg 2100

cgccagggtt acagccccct gagttccag accctgaccc ccaacccccc cgccctggac 2160  
 cgcctggcc gcatcgagga ggaggggcggc gagcaggacc gcgaccgcag catccgcctg 2220  
 gtgcagggtt tcctggccct ggcctggac gacctgcgc gctgtgcct gttcagctac 2280  
 caccgcctgc gecacctgtat cctggtgacc gcccgcgtgg tggagctgtt gggccgcagc 2340  
 agcccccgcg gcttcgcagcg cggctggag gcccgtaaat acctgggcag cctggcgcag 2400  
 tactggggcc ttggactgaa gaagagcgc accagcctgc tggacagcat cgccatcgcc 2460  
 gtggccgagg gcaaccgaccg catcatcgag gtatcccgac gcatctaccg cgcccttcgtc 2520  
 aacatcccccc gccgcgtgcg ccagggcttc gaggccgcccc tgcag 2565

<210> 16  
 <211> 1056  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic a  
 gp41 coding region of HIV strain AF110975

<400> 16  
 gccgtggca tcggcgccgt gatcttcggc ttcttggcg ccggccggcag caacatggc 60  
 gcccgcagca tcaccctgac cgcccagggc cgccagctgc tgagcggcat cgtgcagcag 120  
 cagagaacc tgctgcgcgc catcgagggc cagcagcaca tgctgcagat gaccgtgtgg 180  
 ggcataaggc agctgcaggc ccgcgtgtc gccatcgagc gctacctgaa ggaccaggcag 240  
 ctgctggca tctggggctg cagcgcaag ctgatctgca ccaccaccgt gccctgaaac 300  
 agcagctgga gcaacaagac ccagggcgag atctggaga acatgacctg gatgcagtgg 360  
 gacaaggaga tcagcaacta caccggcattt atctaccggc tgctggagga gagccagaac 420  
 cagcaggagc agaacgagaa ggacctgtg gcccggaca gcccgaacaa cctgtggagc 480  
 tggtaacaaca tcagcaactg gctgtgttac atcaagatct tcatcatgtat cgtggcggc 540  
 ctgatcgcc tcgcgcattat ttgcgcgtg ctgagcatcg tgaaccgcgt ggcgcaggc 600  
 tacagcccccc tgagcttcca gaccctgacc cccaaaaacc gcccggctggc cgcctggc 660  
 cgcacatcgagg aggagggcgg cgagcaggac cgccgaccgc gcatccgcct ggtgcaggc 720  
 ttcttggccc tggctggga cgacctgcgc agcctgtgcc tgttcagcta ccaccgcctg 780  
 cgcgacactga tcctgggtgac cgcccggtg gtggagctgc tggccggcagc cagccccccgc 840  
 ggcctgcgc gcccgtggga gcccctgaaat tacctggca gcttgggtgca gtactgggc 900  
 ctggagctga agaagagcgc caccagcctg ctggacagca tcgccatcg cgtggccgag 960  
 ggcaccgacc gcatcatcgat ggtatcccgac gcatctacc gccccttcgtc caacatcccccc 1020  
 cgcgcgtgc gccagggtt cgaggccgccc ctgcag 1056

<210> 17  
 <211> 492  
 <212> PRT  
 <213> Human immunodeficiency virus

<400> 17  
 Met Gly Ala Arg Ala Ser Ile Leu Arg Gly Gly Lys Leu Asp Ala Trp  
 1 5 10 15

Glu Arg Ile Arg Leu Arg Pro Gly Gly Lys Lys Cys Tyr Met Met Lys  
 20 25 30

His Leu Val Trp Ala Ser Arg Glu Leu Glu Lys Phe Ala Leu Asn Pro  
 35 40 45

Gly Leu Leu Glu Thr Ser Glu Gly Cys Lys Gln Ile Ile Arg Gln Leu  
 50 55 60

His Pro Ala Leu Gln Thr Gly Ser Glu Glu Leu Lys Ser Leu Phe Asn  
 65 70 75 80

Thr Val Ala Thr Leu Tyr Cys Val His Glu Lys Ile Glu Val Arg Asp  
85 90 95

Thr Lys Glu Ala Leu Asp Lys Ile Glu Glu Glu Gln Asn Lys Cys Gln  
100 105 110

Gln Lys Ile Gln Gln Ala Glu Ala Ala Asp Lys Gly Lys Val Ser Gln  
115 120 125

Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala  
130 135 140

Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Ile Glu Glu Lys  
145 150 155 160

Ala Phe Ser Pro Glu Val Ile Pro Met Phe Thr Ala Leu Ser Glu Gly  
165 170 175

Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His  
180 185 190

Gln Ala Ala Met Gln Met Leu Lys Asp Thr Ile Asn Glu Glu Ala Ala  
195 200 205

Glu Trp Asp Arg Val His Pro Val His Ala Gly Pro Ile Ala Pro Gly  
210 215 220

Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr  
225 230 235 240

Leu Gln Glu Gln Ile Ala Trp Met Thr Ser Asn Pro Pro Ile Pro Val  
245 250 255

Gly Asp Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val  
260 265 270

Arg Met Tyr Ser Pro Val Ser Ile Leu Asp Ile Lys Gln Gly Pro Lys  
275 280 285

Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala  
290 295 300

Glu Gln Ser Thr Gln Glu Val Lys Asn Trp Met Thr Asp Thr Leu Leu  
305 310 315 320

Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Arg Ala Leu Gly  
325 330 335

Pro Gly Ala Ser Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly  
340 345 350

Gly Pro Ser His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Ala  
355 360 365

Asn Thr Ser Val Met Met Gln Lys Ser Asn Phe Lys Gly Pro Arg Arg  
370 375 380

Ile Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Arg Asn  
385 390 395 400

Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly  
405 410 415

His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys  
420 425 430

Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg  
435 440 445

Pro Glu Pro Thr Ala Pro Pro Ala Glu Ser Phe Arg Phe Glu Glu Thr  
450 455 460

Thr Pro Gly Gln Lys Gln Glu Ser Lys Asp Arg Glu Thr Leu Thr Ser  
465 470 475 480

Leu Lys Ser Leu Phe Gly Asn Asp Pro Leu Ser Gln  
485 490

<210> 18

<211> 81

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
signal sequence of HIV strain AF110968

<400> 18

atgcgcgtga tggcatcct gaagaactac cagcagtggt ggatgtgggg catcctggc 60  
ttctggatgc tgatcatcag c 81

<210> 19

<211> 72

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic  
signal sequence of HIV strain AF110975

<400> 19

atgcgcgtgc gccccatcct gcgcagctgg cagcagtggt ggatctgggg catcctggc 60  
ttctggatct gc 72

<210> 20

<211> 1479

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: synthetic Gag  
coding sequence of HIV strain AF110965

<400> 20

atggggcccc gcgcagcat cctgcgcggc ggcaagctgg acgcctggga ggcgcattccgc 60  
ctgcgcggcg gcggcaagaa gtgctacatg atgaagcacc tggatgtgggg cagccgcgag 120  
ctggagaagt tcgcctgaa ccccgccctg ctggagacca gcggaggcgtg caagcagatc 180  
atccgcgcgc tgcaccccccgc cctgcagacc ggcagcgagg agctgaagag cctgttcaac 240

accgtggcca ccctgtactg cgtgcacgag aagatcgagg tgcgcgacac caaggaggcc 300  
 ctggacaaga tcgaggagga gcagaacaag tgccagcaga agatccagca ggccgaggcc 360  
 gccgacaagg gcaaggttag ccagaactac cccatcgtgc agaacctgca gggccagatg 420  
 gtgcaccagg ccatcagccc ccgcacccctg aacgcctggg tgaaggtgat cgaggagaag 480  
 gccttcagcc ccgaggtat cccatgttc accgcctgca gcgagggcgc caaaaaaaa 540  
 gacctgaaca ccatgtgaa caccgtggc ggccaccagg ccgcattgca gatgctgaag 600  
 gacaccatca acgaggaggc cgccgagtgg gaccggcgtc accccgtgca cgccggcccc 660  
 atcgcccccg gccagatgca cgagccccgc ggcagcgaca tcgccccgac caccagcacc 720  
 ctgcaggagc agatgcctg gatgaccagc aaaaaaaaaa tccccgtggg cgacatctac 780  
 aagcgctgga tcatcctggg cctgaacaag atcgtgcga tgcatacgccc cgtgagcatc 840  
 ctggacatca agcaggccc caaggagccc ttccgcact acgtggaccg cttttcaag 900  
 accctgcgcg ccgagcagag cacccaggag gtgaagaact ggatgaccga caccctgctg 960  
 gtgcagaacg ccaaaaaaaa ctgcaagacc atcctgcgcg ccctggggcc cggcgccagc 1020  
 ctggaggaga tgcgtgaccgc ctgcccaggcc gtggggccgc ccagccacaa ggcccgctg 1080  
 ctggccgagg ccatgagcca ggccaaacacc agcgtgatga tgcagaagag caacttcaag 1140  
 ggcccccgcgc gcattgtgaa tgcgttcaac tgcggcaagg agggccacat cgcccgcaac 1200  
 tgcggcgccc cccgcaagaa gggctgtgg aagtgcggca aggaggggcca ccagatgaag 1260  
 gactgcaccc agcgccaggc caacttcttgg gcacatgtt gggcccgccca caagggccgc 1320  
 cccggcaact tcctgcagag ccgcggccgg cccaccggcc ccccccggca gagcttcgc 1380  
 ttcgaggaga ccaccccccgg ccagaagcag gagagcaagg accgcgagac cctgaccagc 1440  
 ctgaagagcc tgcgtggcaa cgacccctg agccagtaa 1479

<210> 21  
 <211> 1509  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: synthetic Gag  
 coding sequence of HIV strain AF110967

<400> 21  
 atgggcggcc gggccagcat cctgcgcggc gagaagctgg acaagtgggaa gaagatccgc 60  
 ctgcggcccg gggcaagaa gcaactacatg ctgaagcacc tgggtgtggc cagccgcgag 120  
 ctggagggtc tgcctgtaa ccccgccctg ctggagaccc cgaggggctg caagcagatc 180  
 atgaaggcgc tgcggccgc cctgcacggc ggcacccggg agctgcgcgc cctgtacaac 240  
 accgtggcca ccctgtactg cgtgcacggc ggcacatcgagg tgcgcgcacac caaggaggcc 300  
 ctggacaaaga tcgaggagga gcaacaacaa agccacggaa agacccggca ggccaaaggag 360  
 gcccacggca aggtgagccaa gaaactacccc atcgtgcaga acctgcaggcc ccagatgtg 420  
 caccaggccca tcagcccccg caccctgaaac gcctgggtga aggtgatcga ggagaaggcc 480  
 ttcagcccccg aggtgatcccc catgttccacc gcctggatgc agggcgccac ccccccaggac 540  
 ctgaacacca tgcgttccacc cgtggggccgc caccaggccg ccatgcacat gctgaaggac 600  
 accatcaacg aggaggccgc ctagtgggac cgcctgcacc cctgtgcaggc cggcccccgt 660  
 gccccccggcc agatgcgcga ccccccggccgc agcgacatcg cggccgcac cagcacccctg 720  
 caggagcaga tcgcctggat gaccagcaac ccccccgtgc cctgtgggca catctacaag 780  
 cgctggatca tcctggccct gaacaagatc tgcgcacatg acagccccgt gaggatccctg 840  
 gacatccccc agggccccaa ggagcccttc cgcgactacg tggaccgctt cttcaagacc 900  
 ctgcgcggcc agcaggccac ccaggacgtg aagaacttggaa tgaccggagac cctgtgttg 960  
 cagaacggca accccgactg caagaccatc ctgcgcggcc tggggccccgg cggccacccctg 1020  
 gaggagatga tgaccgcctg ccaggccgtg ggcggccccgg gccacaaggc cccgtgtcg 1080  
 gcccggccca tgagccaggc caacagcgtg aacatcatga tgcagaagag caacttcaag 1140  
 gggcccccggcc gcaacgtgaa gtgttcaac tgcggcaagg agggccacat cggccaaagac 1200  
 tgcgcggccccc cccgcaagaa gggctgtgg aagtgcggca agggaggccca ccagatgaag 1260  
 gactgcaccc agcgccaggc caacttcttgg ggcaagatct gggccacatc caagggccgc 1320  
 cccggcaact tcctgcagaa ccgcacggc gggccggccccc ccacccgtgc caccggcccc 1380  
 cccggccgaga gcttccgttt cgaggagacc acccccccggcc ccaagcagga gccccaaaggac 1440  
 cgcgacccctt accgcgagcc cctgaccggcc ctgcgcagcc tgcgtggcag cggcccccctg 1500  
 agccagtaa 1509

&lt;210&gt; 22

&lt;211&gt; 502

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus

&lt;400&gt; 22

Met	Gly	Ala	Arg	Ala	Ser	Ile	Leu	Arg	Gly	Glu	Lys	Leu	Asp	Lys	Trp
1															
														10	15

Glu	Lys	Ile	Arg	Leu	Arg	Pro	Gly	Gly	Lys	Lys	His	Tyr	Met	Leu	Lys
													20	25	30

His	Leu	Val	Trp	Ala	Ser	Arg	Glu	Leu	Glu	Gly	Phe	Ala	Leu	Asn	Pro
													35	40	45

Gly	Leu	Leu	Glu	Thr	Ala	Glu	Gly	Cys	Lys	Gln	Ile	Met	Lys	Gln	Leu
													50	55	60

Gln	Pro	Ala	Leu	Gln	Thr	Gly	Thr	Glu	Glu	Leu	Arg	Ser	Leu	Tyr	Asn	
													65	70	75	80

Thr	Val	Ala	Thr	Leu	Tyr	Cys	Val	His	Ala	Gly	Ile	Glu	Val	Arg	Asp
													85	90	95

Thr	Lys	Glu	Ala	Leu	Asp	Lys	Ile	Glu	Glu	Glu	Gln	Asn	Lys	Ser	Gln
													100	105	110

Gln	Lys	Thr	Gln	Gln	Ala	Lys	Glu	Ala	Asp	Gly	Lys	Val	Ser	Gln	Asn
													115	120	125

Tyr	Pro	Ile	Val	Gln	Asn	Leu	Gln	Gly	Gln	Met	Val	His	Gln	Ala	Ile
													130	135	140

Ser	Pro	Arg	Thr	Leu	Asn	Ala	Trp	Val	Lys	Val	Ile	Glu	Glu	Lys	Ala	
													145	150	155	160

Phe	Ser	Pro	Glu	Val	Ile	Pro	Met	Phe	Thr	Ala	Leu	Ser	Glu	Gly	Ala
													165	170	175

Thr	Pro	Gln	Asp	Leu	Asn	Thr	Met	Leu	Asn	Thr	Val	Gly	Gly	His	Gln
													180	185	190

Ala	Ala	Met	Gln	Met	Leu	Lys	Asp	Thr	Ile	Asn	Glu	Ala	Ala	Glu	
													195	200	205

Trp	Asp	Arg	Leu	His	Pro	Val	Gln	Ala	Gly	Pro	Val	Ala	Pro	Gly	Gln
													210	215	220

Met	Arg	Asp	Pro	Arg	Gly	Ser	Asp	Ile	Ala	Gly	Ala	Thr	Ser	Thr	Leu	
													225	230	235	240

Gln	Glu	Gln	Ile	Ala	Trp	Met	Thr	Ser	Asn	Pro	Pro	Val	Pro	Val	Gly
													245	250	255

Asp	Ile	Tyr	Lys	Arg	Trp	Ile	Ile	Leu	Gly	Leu	Asn	Lys	Ile	Val	Arg
													260	265	270

Met	Tyr	Ser	Pro	Val	Ser	Ile	Leu	Asp	Ile	Arg	Gln	Gly	Pro	Lys	Glu
													275	280	285

Pro Phe Arg Asp Tyr Val Asp Arg Phe Phe Lys Thr Leu Arg Ala Glu  
 290 295 300

Gln Ala Thr Gln Asp Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val  
 305 310 315 320

Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Arg Ala Leu Gly Pro  
 325 330 335

Gly Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly  
 340 345 350

Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Ala Asn  
 355 360 365

Ser Val Asn Ile Met Met Gln Lys Ser Asn Phe Lys Gly Pro Arg Arg  
 370 375 380

Asn Val Lys Cys Phe Asn Cys Gly Lys Glu Gly His Ile Ala Lys Asn  
 385 390 395 400

Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly  
 405 410 415

His Gln Met Lys Asp Cys Thr Glu Arg Gln Ala Asn Phe Leu Gly Lys  
 420 425 430

Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Asn Arg  
 435 440 445

Ser Glu Pro Ala Ala Pro Thr Val Pro Thr Ala Pro Pro Ala Glu Ser  
 450 455 460

Phe Arg Phe Glu Glu Thr Thr Pro Ala Pro Lys Gln Glu Pro Lys Asp  
 465 470 475 480

Arg Glu Pro Tyr Arg Glu Pro Leu Thr Ala Leu Arg Ser Leu Phe Gly  
 485 490 495

Ser Gly Pro Leu Ser Gln  
 500

<210> 23  
<211> 849  
<212> PRT  
<213> Human immunodeficiency virus

Thr Thr Leu Phe Cys Thr Ser Asp Ala Lys Ala Tyr Glu Thr Glu Val  
 50 55 60

His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro  
 65 70 75 80

Gln Glu Ile Val Leu Glu Asn Val Thr Glu Asn Phe Asn Met Trp Lys  
 85 90 95

Asn Asp Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp  
 100 105 110

Gln Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu  
 115 120 125

Lys Cys Arg Asn Val Asn Ala Thr Asn Asn Ile Asn Ser Met Ile Asp  
 130 135 140

Asn Ser Asn Lys Gly Glu Met Lys Asn Cys Ser Phe Asn Val Thr Thr  
 145 150 155 160

Glu Leu Arg Asp Arg Lys Gln Glu Val His Ala Leu Phe Tyr Arg Leu  
 165 170 175

Asp Val Val Pro Leu Gln Gly Asn Asn Ser Asn Glu Tyr Arg Leu Ile  
 180 185 190

Asn Cys Asn Thr Ser Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe  
 195 200 205

Asp Pro Ile Pro Ile His Tyr Cys Thr Pro Ala Gly Tyr Ala Ile Leu  
 210 215 220

Lys Cys Asn Asn Gln Thr Phe Asn Gly Thr Gly Pro Cys Asn Asn Val  
 225 230 235 240

Ser Ser Val Gln Cys Ala His Gly Ile Lys Pro Val Val Ser Thr Gln  
 245 250 255

Leu Leu Leu Asn Gly Ser Leu Ala Lys Gly Glu Ile Ile Arg Ser  
 260 265 270

Glu Asn Leu Ala Asn Asn Ala Lys Ile Ile Ile Val Gln Leu Asn Lys  
 275 280 285

Pro Val Lys Ile Val Cys Val Arg Pro Asn Asn Asn Thr Arg Lys Ser  
 290 295 300

Val Arg Ile Gly Pro Gly Gln Thr Phe Tyr Ala Thr Gly Glu Ile Ile  
 305 310 315 320

Gly Asp Ile Arg Gln Ala Tyr Cys Ile Ile Asn Lys Thr Glu Trp Asn  
 325 330 335

Ser Thr Leu Gln Gly Val Ser Lys Lys Leu Glu Glu His Phe Ser Lys  
 340 345 350

Lys Ala Ile Lys Phe Glu Pro Ser Ser Gly Gly Asp Leu Glu Ile Thr  
 355 360 365

Thr His Ser Phe Asn Cys Arg Gly Glu Phe Phe Tyr Cys Asp Thr Ser  
 370                   375                   380  
  
 Gln Leu Phe Asn Ser Thr Tyr Ser Pro Ser Phe Asn Gly Thr Glu Asn  
 385                   390                   395                   400  
  
 Lys Leu Asn Gly Thr Ile Thr Ile Thr Cys Arg Ile Lys Gln Ile Ile  
 405                   410                   415  
  
 Asn Met Trp Gln Lys Val Gly Arg Ala Met Tyr Ala Pro Pro Ile Ala  
 420                   425                   430  
  
 Gly Asn Leu Thr Cys Glu Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg  
 435                   440                   445  
  
 Asp Gly Gly Lys Thr Gly Pro Asn Asp Thr Glu Ile Phe Arg Pro Gly  
 450                   455                   460  
  
 Gly Gly Asp Met Arg Asp Asn Trp Arg Asn Glu Leu Tyr Lys Tyr Lys  
 465                   470                   475                   480  
  
 Val Val Glu Ile Lys Pro Leu Gly Val Ala Pro Thr Glu Ala Lys Arg  
 485                   490                   495  
  
 Arg Val Val Glu Arg Glu Lys Arg Ala Val Gly Ile Gly Ala Val Phe  
 500                   505                   510  
  
 Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala Ala Ser Ile  
 515                   520                   525  
  
 Thr Leu Thr Val Gln Ala Arg Leu Leu Leu Ser Gly Ile Val Gln Gln  
 530                   535                   540  
  
 Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Leu Leu Gln  
 545                   550                   555                   560  
  
 Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Thr Arg Ile Leu Ala Val  
 565                   570                   575  
  
 Glu Arg Tyr Leu Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser  
 580                   585                   590  
  
 Gly Lys Leu Ile Cys Thr Thr Ala Val Pro Trp Asn Ser Ser Trp Ser  
 595                   600                   605  
  
 Asn Arg Ser His Asp Glu Ile Trp Asp Asn Met Thr Trp Met Gln Trp  
 610                   615                   620  
  
 Asp Arg Glu Ile Asn Asn Tyr Thr Asp Thr Ile Tyr Arg Leu Leu Glu  
 625                   630                   635                   640  
  
 Glu Ser Gln Asn Gln Gln Glu Lys Asn Glu Lys Asp Leu Leu Ala Leu  
 645                   650                   655  
  
 Asp Ser Trp Gln Asn Leu Trp Asn Trp Phe Ser Ile Thr Asn Trp Leu  
 660                   665                   670  
  
 Trp Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu Ile Gly Leu  
 675                   680                   685

Arg Ile Ile Phe Ala Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly  
 690                    695                    700

Tyr Ser Pro Leu Pro Phe Gln Thr Leu Thr Pro Asn Pro Arg Glu Pro  
 705                    710                    715                    720

Asp Arg Leu Gly Arg Ile Glu Glu Gly Gly Glu Gln Asp Arg Gly  
 725                    730                    735

Arg Ser Ile Arg Leu Val Ser Gly Phe Leu Ala Leu Ala Trp Asp Asp  
 740                    745                    750

Leu Arg Ser Leu Cys Leu Phe Ser Tyr His Arg Leu Arg Asp Phe Ile  
 755                    760                    765

Leu Ile Ala Ala Arg Val Leu Glu Leu Leu Gly Gln Arg Gly Trp Glu  
 770                    775                    780

Ala Leu Lys Tyr Leu Gly Ser Leu Val Gln Tyr Trp Gly Leu Glu Leu  
 785                    790                    795                    800

Lys Lys Ser Ala Ile Ser Leu Leu Asp Thr Ile Ala Ile Ala Val Ala  
 805                    810                    815

Glu Gly Thr Asp Arg Ile Ile Glu Phe Ile Gln Arg Ile Cys Arg Ala  
 820                    825                    830

Ile Arg Asn Ile Pro Arg Arg Ile Arg Gln Gly Phe Glu Ala Ala Leu  
 835                    840                    845

Gln

<210> 24

<211> 855

<212> PRT

<213> Human immunodeficiency virus

<400> 24

Met Arg Val Arg Gly Ile Leu Arg Ser Trp Gln Gln Trp Trp Ile Trp  
 1                    5                    10                    15

Gly Ile Leu Gly Phe Trp Ile Cys Ser Gly Leu Gly Asn Leu Trp Val  
 20                    25                    30

Thr Val Tyr Asp Gly Val Pro Val Trp Arg Glu Ala Ser Thr Thr Leu  
 35                    40                    45

Phe Cys Ala Ser Asp Ala Lys Ala Tyr Glu Lys Glu Val His Asn Val  
 50                    55                    60

Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro Gln Glu Ile  
 65                    70                    75                    80

Glu Leu Asp Asn Val Thr Glu Asn Phe Asn Met Trp Lys Asn Asp Met  
 85                    90                    95

Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp Gln Ser Leu  
100 105 110

Lys Pro Arg Val Lys Leu Thr Pro Leu Cys Val Thr Leu Lys Cys Thr  
115 120 125

Asn Tyr Ser Thr Asn Tyr Ser Asn Thr Met Asn Ala Thr Ser Tyr Asn  
130 135 140

Asn Asn Thr Thr Glu Glu Ile Lys Asn Cys Thr Phe Asn Met Thr Thr  
145 150 155 160

Glu Leu Arg Asp Lys Lys Gln Gln Val Tyr Ala Leu Phe Tyr Lys Leu  
165 170 175

Asp Ile Val Pro Leu Asn Ser Asn Ser Ser Glu Tyr Arg Leu Ile Asn  
180 185 190

Cys Asn Thr Ser Ala Ile Thr Gln Ala Cys Pro Lys Val Ser Phe Asp  
195 200 205

Pro Ile Pro Ile His Tyr Cys Ala Pro Ala Gly Tyr Ala Ile Leu Lys  
210 215 220

Cys Lys Asn Asn Thr Ser Asn Gly Thr Gly Pro Cys Gln Asn Val Ser  
225 230 235 240

Thr Val Gln Cys Thr His Gly Ile Lys Pro Val Val Ser Thr Pro Leu  
245 250 255

Leu Leu Asn Gly Ser Leu Ala Glu Gly Gly Glu Ile Ile Ile Arg Ser  
260 265 270

Lys Asn Leu Ser Asn Asn Ala Tyr Thr Ile Ile Val His Leu Asn Asp  
275 280 285

Ser Val Glu Ile Val Cys Thr Arg Pro Asn Asn Asn Thr Arg Lys Gly  
290 295 300

Ile Arg Ile Gly Pro Gly Gln Thr Phe Tyr Ala Thr Glu Asn Ile Ile  
305 310 315 320

Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Ala Gly Glu Trp Asn  
325 330 335

Lys Ala Val Gln Arg Val Ser Ala Lys Leu Arg Glu His Phe Pro Asn  
340 345 350

Lys Thr Ile Glu Phe Gln Pro Ser Ser Gly Gly Asp Leu Glu Ile Thr  
355 360 365

Thr His Ser Phe Asn Cys Arg Gly Glu Phe Phe Tyr Cys Asn Thr Ser  
370 375 380

Lys Leu Phe Asn Ser Ser Tyr Asn Gly Thr Ser Tyr Arg Gly Thr Glu  
385 390 395 400

Ser Asn Ser Ser Ile Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile  
405 410 415

Asp Met Trp Gln Lys Val Gly Arg Ala Ile Tyr Ala Pro Pro Ile Glu  
 420                    425                    430

Gly Asn Ile Thr Cys Ser Ser Ser Ile Thr Gly Leu Leu Leu Ala Arg  
 435                    440                    445

Asp Gly Gly Leu Asp Asn Ile Thr Thr Glu Ile Phe Arg Pro Gln Gly  
 450                    455                    460

Gly Asp Met Lys Asp Asn Trp Arg Asn Glu Leu Tyr Lys Tyr Lys Val  
 465                    470                    475                    480

Val Glu Ile Lys Pro Leu Gly Val Ala Pro Thr Glu Ala Lys Arg Arg  
 485                    490                    495

Val Val Glu Arg Glu Lys Arg Ala Val Gly Ile Gly Ala Val Ile Phe  
 500                    505                    510

Gly Phe Leu Gly Ala Ala Gly Ser Asn Met Gly Ala Ala Ser Ile Thr  
 515                    520                    525

Leu Thr Ala Gln Ala Arg Gln Leu Leu Ser Gly Ile Val Gln Gln Gln  
 530                    535                    540

Ser Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Met Leu Gln Leu  
 545                    550                    555                    560

Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val Leu Ala Ile Glu  
 565                    570                    575

Arg Tyr Leu Lys Asp Gln Gln Leu Leu Gly Ile Trp Gly Cys Ser Gly  
 580                    585                    590

Lys Leu Ile Cys Thr Thr Val Pro Trp Asn Ser Ser Trp Ser Asn  
 595                    600                    605

Lys Thr Gln Gly Glu Ile Trp Glu Asn Met Thr Trp Met Gln Trp Asp  
 610                    615                    620

Lys Glu Ile Ser Asn Tyr Thr Gly Ile Ile Tyr Arg Leu Leu Glu Glu  
 625                    630                    635                    640

Ser Gln Asn Gln Glu Gln Asn Glu Lys Asp Leu Leu Ala Leu Asp  
 645                    650                    655

Ser Arg Asn Asn Leu Trp Ser Trp Phe Asn Ile Ser Asn Trp Leu Trp  
 660                    665                    670

Tyr Ile Lys Ile Phe Ile Met Ile Val Gly Gly Leu Ile Gly Leu Arg  
 675                    680                    685

Ile Ile Phe Ala Val Leu Ser Ile Val Asn Arg Val Arg Gln Gly Tyr  
 690                    695                    700

Ser Pro Leu Ser Phe Gln Thr Leu Thr Pro Asn Pro Arg Gly Leu Asp  
 705                    710                    715                    720

Arg Leu Gly Arg Ile Glu Glu Glu Gly Glu Gln Asp Arg Asp Arg  
 725                    730                    735

Ser Ile Arg Leu Val Gln Gly Phe Leu Ala Leu Ala Trp Asp Asp Leu  
740 745 750

Arg Ser Leu Cys Leu Phe Ser Tyr His Arg Leu Arg Asp Leu Ile Leu  
755 760 765

Val Thr Ala Arg Val Val Glu Leu Leu Gly Arg Ser Ser Pro Arg Gly  
770 775 780

Leu Gln Arg Gly Trp Glu Ala Leu Lys Tyr Leu Gly Ser Leu Val Gln  
785 790 795 800

Tyr Trp Gly Leu Glu Leu Lys Lys Ser Ala Thr Ser Leu Leu Asp Ser  
805 810 815

Ile Ala Ile Ala Val Ala Glu Gly Thr Asp Arg Ile Ile Glu Val Ile  
820 825 830

Gln Arg Ile Tyr Arg Ala Phe Cys Asn Ile Pro Arg Arg Val Arg Gln  
835 840 845

Gly Phe Glu Ala Ala Leu Gln  
850 855

<210> 25

<211> 20

<212> PRT

<213> Human immunodeficiency virus

<400> 25

Asp Ile Lys Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg  
1 5 10 15

Phe Phe Lys Thr  
20

<210> 26

<211> 60

<212> DNA

<213> Human immunodeficiency virus

<400> 26

gacataaaac aaggacccaaa agagcccttt agagactatg tagaccgggtt ctttaaaacc 60

<210> 27

<211> 20

<212> PRT

<213> Human immunodeficiency virus

<400> 27

Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg  
1 5 10 15

Phe Phe Lys Thr  
20

&lt;210&gt; 28

&lt;211&gt; 47

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus

&lt;400&gt; 28

Thr Ile Thr Ile Thr Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln  
1 5 10 15

Lys Val Gly Arg Ala Met Tyr Ala Pro Pro Ile Ala Gly Asn Leu Thr  
20 25 30

Cys Glu Ser Asn Ile Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly  
35 40 45

&lt;210&gt; 29

&lt;211&gt; 48

&lt;212&gt; PRT

&lt;213&gt; Human immunodeficiency virus

&lt;400&gt; 29

Ser Ile Ile Thr Leu Pro Cys Arg Ile Lys Gln Ile Ile Asp Met Trp  
1 5 10 15

Gln Lys Val Gly Arg Ala Ile Tyr Ala Pro Pro Ile Glu Gly Asn Ile  
20 25 30

Thr Cys Ser Ser Ser Ile Thr Gly Leu Leu Leu Ala Arg Asp Gly Gly  
35 40 45